

A62

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization International Bureau



(43) International Publication Date
13 May 2004 (13.05.2004)

PCT

(10) International Publication Number
WO 2004/039950 A2

(51) International Patent Classification⁷:

C12N

Emeryville, CA 94662-8097 (US). SELBY, Mark [US/US]; c/o Chiron Corporation, P.O. Box 8097, Emeryville, CA 94662-8097 (US). PALIARD, Xavier [US/US]; c/o Chiron Corporation, P.O. Box 8097, Emeryville, CA 94662-8097 (US).

(21) International Application Number:

PCT/US2003/033610

(74) Agents: HARBIN, Alisa et al.; Chiron Corporation, Intellectual Property - R440, P.O. Box 8097, Emeryville, CA 94662-8097 (US).

(22) International Filing Date: 24 October 2003 (24.10.2003)

(25) Filing Language:

English

(26) Publication Language:

English

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(30) Priority Data:

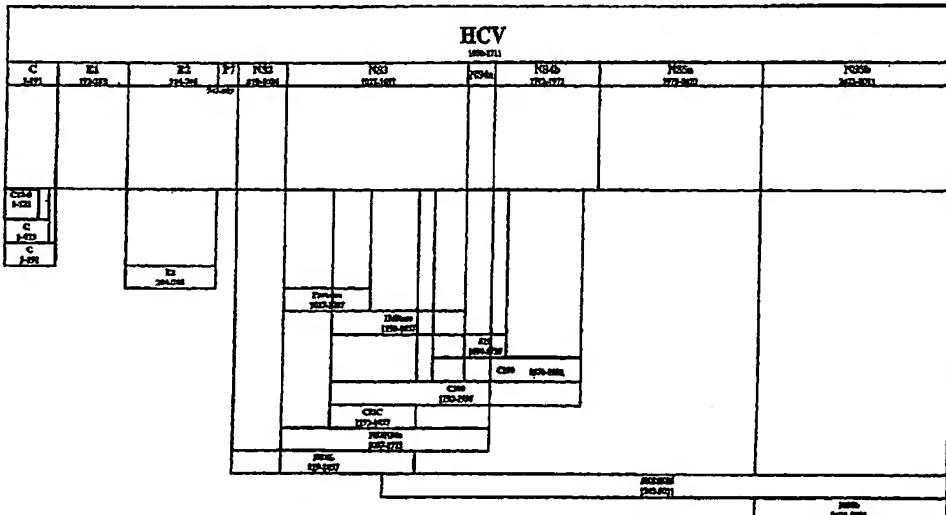
10/281,341 25 October 2002 (25.10.2002) US

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO,

[Continued on next page]

(54) Title: ACTIVATION OF HCV-SPECIFIC CELLS

HCV Genome and Recombinant Proteins



(57) Abstract: The invention provides a method of activating hepatitis C virus (HCV)-specific T cells, including CD4⁺ and CD8⁺ T cells. HCV-specific T cells are activated using fusion proteins comprising HCV NS3, NS4, NS5a, and NS5b polypeptides, polynucleotides encoding such fusion proteins, or polypeptide or polynucleotide compositions containing the individual components of these fusions. The method can be used in model systems to develop HCV-specific immunogenic compositions, as well as to immunize a mammal against HCV.

WO 2004/039950 A2



SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Published:

- *without international search report and to be republished upon receipt of that report*

5

ACTIVATION OF HCV-SPECIFIC T CELLS

TECHNICAL AREA OF THE INVENTION

10 The invention relates to the activation of hepatitis C virus(HCV)-specific T cells. More particularly, the invention relates to the use of multiple HCV polypeptides, either alone or as fusions, to stimulate cell-mediated immune responses, such as to activate HCV-specific T cells.

15 BACKGROUND OF THE INVENTION

Hepatitis C virus (HCV) infection is an important health problem with approximately 1% of the world's population infected with the virus. Over 75% of acutely infected individuals eventually progress to a chronic carrier state that can result in cirrhosis, liver failure, and hepatocellular carcinoma. *See* Alter *et al.* (1992)

20 N. Engl. J. Med. 327:1899-1905; Resnick and Koff. (1993) Arch. Intern. Med. 153:1672-1677; Seeff (1995) Gastrointest. Dis. 6:20-27; Tong *et al.* (1995) N. Engl. J. Med. 332:1463-1466.

25 Despite extensive advances in the development of pharmaceuticals against certain viruses like HIV, control of acute and chronic HCV infection has had limited success (Hoofnagle and di Bisceglie (1997) N. Engl. J. Med. 336:347-356). In particular, generation of a strong cytotoxic T lymphocyte (CTL) response is thought to be important for the control and eradication of HCV infections. Thus, there is a need in the art for effective methods of inducing strong CTL responses against HCV.

SUMMARY OF THE INVENTION

It is an object of the invention to provide reagents and methods for stimulating immune responses, such as activating T cells which recognize epitopes of HCV polypeptides. This and other objects of the invention are provided by one or more of 5 the embodiments described below.

The invention provides HCV proteins useful for stimulating immune responses, such as activating HCV-specific T cells. One embodiment provides a fusion protein that comprises HCV polypeptides, wherein the HCV polypeptides consist essentially of an NS3, an NS4, an NS5a polypeptide, and optionally a core 10 polypeptide. In certain embodiments, the fusion protein includes an NS5b polypeptide.

In certain embodiments, at least one of the HCV polypeptides is derived from a different strain of HCV than the other polypeptides.

The invention also provides compositions comprising any of these fusion 15 proteins and a pharmaceutically acceptable excipient. In certain embodiments, the compositions further comprise an adjuvant, a CpG polynucleotide and/or the fusion protein is adsorbed to or entrapped within a microparticle or ISCOM. The compositions can further comprise a polynucleotide encoding an E1E2 complex. The E1E2 polynucleotide can also be adsorbed to or entrapped withing a microparticle.

Another embodiment provides a composition comprising HCV polypeptides and a pharmaceutically acceptable excipient. The HCV polypeptides consist 20 essentially of an NS3, an NS4, an NS5a polypeptide, and optionally a core polypeptide. In certain embodiments, the composition includes an NS5b polypeptide. In other embodiments, the compositions further comprise an adjuvant, a CpG 25 polynucleotide and/or one or more of the HCV polypeptides is adsorbed to or entrapped within a microparticle or ISCOM. The compositions can further comprise a polynucleotide encoding an E1E2 complex. The E1E2 polynucleotide can also be adsorbed to or entrapped withing a microparticle.

Moreover, one of the HCV polypeptides may be derived from a different strain of 30 HCV than the others.

Even another embodiment of the invention provides an isolated and purified polynucleotide which encodes a fusion protein as described above. In additional embodiments, the fusion proteins further include a polynucleotide encoding an E1E2 complex.

5 Yet another embodiment of the invention provides a composition comprising the polynucleotides described above and a pharmaceutically acceptable excipient. In certain embodiments, the compositions further comprise an adjuvant and/or the polynucleotide may be adsorbed to or entrapped within a microparticle. The compositions can further comprise a polynucleotide encoding an E1E2 complex. The 10 E1E2 polynucleotide can also be adsorbed to or entrapped within a microparticle.

In a further embodiment, the invention provides a composition comprising HCV polynucleotides and a pharmaceutically acceptable excipient, wherein the HCV polynucleotides consist essentially of polynucleotides encoding an NS3, an NS4, an NS5a polypeptide, and optionally a core polypeptide. In certain embodiments, the 15 composition also includes a polynucleotide encoding an NS5b polypeptide. The compositions may further comprise an adjuvant and/or one or more of the polynucleotides may be adsorbed to or entrapped within a microparticle. The compositions can further comprise a polynucleotide encoding an E1E2 complex. The E1E2 polynucleotide can also be adsorbed to or entrapped within a microparticle. The 20 Additionally, one or more of the polynucleotides may be derived from a different strain of HCV than the others.

In another embodiment, the invention provides a method of activating T cells which recognize an epitope of an HCV polypeptide. T cells are contacted with any of the fusions, polynucleotides or compositions described above. A population of 25 activated T cells recognizes an epitope of the NS3, NS4, NS5a, NS5b, core and/or E1E2 polypeptide.

In the proteins and polynucleotides above, the regions in the fusions need not be in the order in which they naturally occur in the native HCV polyprotein. Thus, for example, the NS5b polypeptide, if present, may be at the N- and/or C-terminus of the 30 fusion, or may be located internally. Similarly, the E1 polypeptide may precede or follow the E2 polypeptide. The E1E2 polypeptide may also be part of the

nonstructural fusion protein or may be provided separately, as an E1E2 complex, or as individual polypeptides.

Moreover, the NS3 polypeptide may include a modification to inhibit protease activity, such that cleavage of the fusion is inhibited. Such modifications are 5 described more fully below. Additionally, the compositions can comprise more than one HCV nonstructural fusion protein, such as a fusion protein with NS3, NS4 and NS5a, and a fusion protein with NS3, NS4, NS5a, NS5b and E1E2. The E1E2 complexes, whether present separately or as part of the fusion, can have varying E1E2 polypeptides (described more fully below).

10 In certain embodiments, the nonstructural fusion protein consists of, from the amino terminus to the carboxyl terminus, an NS3, an NS4, an NS5a and, optionally, an NS5b polypeptide and the E1E2 complex consists of, from amino terminus to the carboxyl terminus, an E1 polypeptide and an E2 polypeptide.

15 The various polypeptides (and polynucleotides encoding therefor) are derived from the same HCV isolate, or from different strains and isolates including isolates having any of the various HCV genotypes, to provide increased protection against a broad range of HCV genotypes.

20 Yet another embodiment of the invention provides a method of stimulating an immune response, such as a cellular immune response, in a vertebrate subject by administering a composition as described herein. In certain embodiments, the composition activates T cells which recognize an epitope of an HCV polypeptide. T cells are contacted with a composition as described above. A population of activated T cells recognizes an epitope of one or more of the HCV polypeptide(s).

25 The invention thus provides methods and reagents for stimulating immune responses to HCV, such as for activating T cells which recognize epitopes of HCV polypeptides. These methods and reagents are particularly advantageous for identifying epitopes of HCV polypeptides associated with a strong CTL response and for immunizing mammals, including humans, against HCV.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a diagrammatic representation of the HCV genome, depicting the various regions of the HCV polyprotein.

5 Figure 2 (SEQ ID NOS: 9 and 10) depicts the DNA and corresponding amino acid sequence of a representative native NS3 protease domain.

Figures 3A-3C (SEQ ID NOS:3 and 4) shows the nucleotide and corresponding amino acid sequence for the HCV-1 E1/E2/p7 region. The numbers shown in the figure are relative to the full-length HCV-1 polyprotein. The E1, E2 and p7 regions are shown.

10 Figure 4 is a diagram of plasmid pMHE1E2-809, encoding E1E2₈₀₉, a representative E1E2 protein for use with the present invention.

Figures 5A-5J (SEQ ID NOS:7 and 8) depict the DNA and corresponding amino acid sequence of a representative NS345Core fusion protein. The depicted sequence includes amino acids 1242-3011 of the HCV polyprotein (representing 15 polypeptides from NS3, NS4, NS5a and NS5b) with amino acids 1-121 of the HCV polyprotein (representing a polypeptide from the core region) fused to the C-terminus of NS5b. This numbering is relative to the HCV-1 polyprotein.

Figure 6 shows a side-by-side comparison of IFN- γ expression generated in animals in response to delivery of alphavirus constructs encoding NS3NS4NS5a.

20 Figure 7 shows IFN- γ expression generated in animals in response to delivery of plasmid DNA encoding NS3NS4NS5a ("naked"), PLG-linked DNA encoding NS3NS4NS5a ("PLG"), separate DNA plasmids encoding NS5a, NS34a, and NS4ab ("naked"), and PLG-linked DNA encoding NS5a, NS34a, and NS4ab ("PLG").

25 Figure 8 shows HCV-specific CD8+ and CD4+ responses in vaccinated chimpanzees.

Figure 9 depicts the specificity of T cell responses primed by electroporation of plasmid DNA two weeks subsequent to the third immunization.

30 Figure 10 shows the specificity of T cell responses primed by vaccinating chimpanzees with NS345Core₁₂₁-ISCOMS two weeks subsequent to the third immunization.

DETAILED DESCRIPTION OF THE INVENTION

The practice of the present invention will employ, unless otherwise indicated, conventional methods of chemistry, biochemistry, recombinant DNA techniques and immunology, within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook, et al., *Molecular Cloning: A Laboratory Manual* (2nd Edition); *Methods In Enzymology* (S. Colowick and N. Kaplan eds., Academic Press, Inc.); *DNA Cloning*, Vols. I and II (D.N. Glover ed.); *Oligonucleotide Synthesis* (M.J. Gait ed.); *Nucleic Acid Hybridization* (B.D. Hames & S.J. Higgins eds.); *Animal Cell Culture* (R.K. Freshney ed.); Perbal, B., *A Practical Guide to Molecular Cloning*.

It must be noted that, as used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "an antigen" includes a mixture of two or more antigens, and the like.

The following amino acid abbreviations are used throughout the text:

15	Alanine: Ala (A)	Arginine: Arg (R)
	Asparagine: Asn (N)	Aspartic acid: Asp (D)
	Cysteine: Cys (C)	Glutamine: Gln (Q)
	Glutamic acid: Glu (E)	Glycine: Gly (G)
	Histidine: His (H)	Isoleucine: Ile (I)
20	Leucine: Leu (L)	Lysine: Lys (K)
	Methionine: Met (M)	Phenylalanine: Phe (F)
	Proline: Pro (P)	Serine: Ser (S)
	Threonine: Thr (T)	Tryptophan: Trp (W)
	Tyrosine: Tyr (Y)	Valine: Val (V)

25

I. Definitions

In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below.

The terms "polypeptide" and "protein" refer to a polymer of amino acid residues and are not limited to a minimum length of the product. Thus, peptides, oligopeptides, dimers, multimers, and the like, are included within the definition.

Both full-length proteins and fragments thereof are encompassed by the definition. The terms also include postexpression modifications of the polypeptide, for example, glycosylation, acetylation, phosphorylation and the like. Furthermore, for purposes of the present invention, a "polypeptide" refers to a protein which includes

5 modifications, such as deletions, additions and substitutions (generally conservative in nature), to the native sequence, so long as the protein maintains the desired activity. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts which produce the proteins or errors due to PCR amplification.

10 An HCV polypeptide is a polypeptide, as defined above, derived from the HCV polyprotein. The polypeptide need not be physically derived from HCV, but may be synthetically or recombinantly produced. Moreover, the polypeptide may be derived from any of the various HCV strains and isolates including isolates having any of the 6 genotypes of HCV described in Simmonds et al., *J. Gen. Virol.* (1993)

15 74:2391-2399 (e.g., strains 1, 2, 3, 4 etc.), as well as newly identified isolates, and subtypes of these isolates, such as HCV1a, HCV1b, etc. A number of conserved and variable regions are known between these strains and, in general, the amino acid sequences of epitopes derived from these regions will have a high degree of sequence homology, e.g., amino acid sequence homology of more than 30%, preferably more

20 than 40%, when the two sequences are aligned. Thus, for example, the term "NS4" polypeptide refers to native NS4 from any of the various HCV strains, as well as NS4 analogs, muteins and immunogenic fragments, as defined further below.

By an "E1 polypeptide" is meant a molecule derived from an HCV E1 region. The mature E1 region of HCV-1 begins at approximately amino acid 192 of the

25 polyprotein and continues to approximately amino acid 383, numbered relative to the full-length HCV-1 polyprotein. (See, Figures 1 and 3A-3C. Amino acids 192-383 of Figures 3A-3C correspond to amino acid positions 20-211 of SEQ ID NO:4.) Amino acids at around 173 through approximately 191 (amino acids 1-19 of SEQ ID NO: 4) serve as a signal sequence for E1. Thus, by an "E1 polypeptide" is meant either a

30 precursor E1 protein, including the signal sequence, or a mature E1 polypeptide which lacks this sequence, or even an E1 polypeptide with a heterologous signal sequence.

The E1 polypeptide includes a C-terminal membrane anchor sequence which occurs at approximately amino acid positions 360-383 (see, International Publication No. WO 96/04301, published February 15, 1996). An E1 polypeptide, as defined herein, may or may not include the C-terminal anchor sequence or portions thereof.

5 By an "E2 polypeptide" is meant a molecule derived from an HCV E2 region. The mature E2 region of HCV-1 begins at approximately amino acid 383-385, numbered relative to the full-length HCV-1 polyprotein. (See, Figures 1 and 3A-3C. Amino acids 383-385 of Figures 3A-3C correspond to amino acid positions 211-213 of SEQ ID NO:4.) A signal peptide begins at approximately amino acid 364 of the 10 polyprotein. Thus, by an "E2 polypeptide" is meant either a precursor E2 protein, including the signal sequence, or a mature E2 polypeptide which lacks this sequence, or even an E2 polypeptide with a heterologous signal sequence. The E2 polypeptide includes a C-terminal membrane anchor sequence which occurs at approximately amino acid positions 715-730 and may extend as far as approximately amino acid 15 residue 746 (see, Lin et al., *J. Virol.* (1994) 68:5063-5073). An E2 polypeptide, as defined herein, may or may not include the C-terminal anchor sequence or portions thereof. Moreover, an E2 polypeptide may also include all or a portion of the p7 region which occurs immediately adjacent to the C-terminus of E2. As shown in Figures 1 and 3A-3C, the p7 region is found at positions 747-809, numbered relative 20 to the full-length HCV-1 polyprotein (amino acid positions 575-637 of SEQ ID NO:4). Additionally, it is known that multiple species of HCV E2 exist (Spaete et al., *Virol.* (1992) 188:819-830; Selby et al., *J. Virol.* (1996) 70:5177-5182; Grakoui et al., *J. Virol.* (1993) 67:1385-1395; Tomei et al., *J. Virol.* (1993) 67:4017-4026). Accordingly, for purposes of the present invention, the term "E2" encompasses any of 25 these species of E2 including, without limitation, species that have deletions of 1-20 or more of the amino acids from the N-terminus of the E2, such as, e.g., deletions of 1, 2, 3, 4, 5...10...15, 16, 17, 18, 19... etc. amino acids. Such E2 species include those beginning at amino acid 387, amino acid 402, amino acid 403, etc.

Representative E1 and E2 regions from HCV-1 are shown in Figures 3A-3C 30 and SEQ ID NO:4. For purposes of the present invention, the E1 and E2 regions are defined with respect to the amino acid number of the polyprotein encoded by the

genome of HCV-1, with the initiator methionine being designated position 1. See, e.g., Choo et al., *Proc. Natl. Acad. Sci. USA* (1991) 88:2451-2455. However, it should be noted that the term an "E1 polypeptide" or an "E2 polypeptide" as used herein is not limited to the HCV-1 sequence. In this regard, the corresponding E1 or 5 E2 regions in other HCV isolates can be readily determined by aligning sequences from the isolates in a manner that brings the sequences into maximum alignment. This can be performed with any of a number of computer software packages, such as ALIGN 1.0, available from the University of Virginia, Department of Biochemistry (Attn: Dr. William R. Pearson). See, Pearson et al., *Proc. Natl. Acad. Sci. USA* (1988) 10 85:2444-2448.

Furthermore, an "E1 polypeptide" or an "E2 polypeptide" as defined herein is not limited to a polypeptide having the exact sequence depicted in the Figures. Indeed, the HCV genome is in a state of constant flux *in vivo* and contains several variable domains which exhibit relatively high degrees of variability between isolates. 15 A number of conserved and variable regions are known between these strains and, in general, the amino acid sequences of epitopes derived from these regions will have a high degree of sequence homology, e.g., amino acid sequence homology of more than 30%, preferably more than 40%, more than 60%, and even more than 80-90% homology, when the two sequences are aligned. It is readily apparent that the terms 20 encompass E1 and E2 polypeptides from any of the various HCV strains and isolates including isolates having any of the 6 genotypes of HCV described in Simmonds et al., *J. Gen. Virol.* (1993) 74:2391-2399 (e.g., strains 1, 2, 3, 4 etc.), as well as newly identified isolates, and subtypes of these isolates, such as HCV1a, HCV1b etc.

Thus, for example, the term "E1" or "E2" polypeptide refers to native E1 or E2 25 sequences from any of the various HCV strains, as well as analogs, muteins and immunogenic fragments, as defined further below. The complete genotypes of many of these strains are known. See, e.g., U.S. Patent No. 6,150,087 and GenBank Accession Nos. AJ238800 and AJ238799.

Additionally, the terms "E1 polypeptide" and "E2 polypeptide" encompass 30 proteins which include modifications to the native sequence, such as internal deletions, additions and substitutions (generally conservative in nature). These

modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through naturally occurring mutational events. All of these modifications are encompassed in the present invention so long as the modified E1 and E2 polypeptides function for their intended purpose. Thus, for example, if the E1 and/or E2 polypeptides are to be used in vaccine compositions, the modifications must be such that immunological activity (i.e., the ability to elicit a humoral or cellular immune response to the polypeptide) is not lost.

5 By "E1E2" complex is meant a protein containing at least one E1 polypeptide and at least one E2 polypeptide, as described above. Such a complex may also include all or a portion of the p7 region which occurs immediately adjacent to the C-terminus of E2. As shown in Figures 1 and 3A-3C, the p7 region is found at positions 747-809, numbered relative to the full-length HCV-1 polyprotein (amino acid positions 575-637 of SEQ ID NO:4). A representative E1E2 complex which includes the p7 protein is termed "E1E2₈₀₉" herein.

10 15 The mode of association of E1 and E2 in an E1E2 complex is immaterial. The E1 and E2 polypeptides may be associated through non-covalent interactions such as through electrostatic forces, or by covalent bonds. For example, the E1E2 polypeptides of the present invention may be in the form of a fusion protein which includes an immunogenic E1 polypeptide and an immunogenic E2 polypeptide, as defined above. The fusion may be expressed from a polynucleotide encoding an E1E2 chimera. Alternatively, E1E2 complexes may form spontaneously simply by mixing E1 and E2 proteins which have been produced individually. Similarly, when co-expressed and secreted into media, the E1 and E2 proteins can form a complex spontaneously. Thus, the term encompasses E1E2 complexes (also called aggregates) 20 25 that spontaneously form upon purification of E1 and/or E2. Such aggregates may include one or more E1 monomers in association with one or more E2 monomers. The number of E1 and E2 monomers present need not be equal so long as at least one E1 monomer and one E2 monomer are present. Detection of the presence of an E1E2 complex is readily determined using standard protein detection techniques such as 30 polyacrylamide gel electrophoresis and immunological techniques such as immunoprecipitation.

The terms "analog" and "mutein" refer to biologically active derivatives of the reference molecule, or fragments of such derivatives, that retain desired activity, such as the ability to stimulate a cell-mediated immune response, as defined below. In general, the term "analog" refers to compounds having a native polypeptide sequence and structure with one or more amino acid additions, substitutions (generally conservative in nature) and/or deletions, relative to the native molecule, so long as the modifications do not destroy immunogenic activity. The term "mutein" refers to peptides having one or more peptide mimics ("peptoids"), such as those described in International Publication No. WO 91/04282. Preferably, the analog or mutein has at least the same immunoactivity as the native molecule. Methods for making polypeptide analogs and muteins are known in the art and are described further below.

As explained above, analogs generally include substitutions that are conservative in nature, i.e., those substitutions that take place within a family of amino acids that are related in their side chains. Specifically, amino acids are generally divided into four families: (1) acidic -- aspartate and glutamate; (2) basic -- lysine, arginine, histidine; (3) non-polar -- alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar -- glycine, asparagine, glutamine, cysteine, serine threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified as aromatic amino acids. For example, it is reasonably predictable that an isolated replacement of leucine with isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar conservative replacement of an amino acid with a structurally related amino acid, will not have a major effect on the biological activity. For example, the polypeptide of interest may include up to about 5-10 conservative or non-conservative amino acid substitutions, or even up to about 15-25 conservative or non-conservative amino acid substitutions, or any integer between 5-25, so long as the desired function of the molecule remains intact. One of skill in the art may readily determine regions of the molecule of interest that can tolerate change by reference to Hopp/Woods and Kyte-Doolittle plots, well known in the art.

By "modified NS3" is meant an NS3 polypeptide with a modification such that protease activity of the NS3 polypeptide is disrupted. The modification can include one or more amino acid additions, substitutions (generally non-conservative in nature) and/or deletions, relative to the native molecule, wherein the protease activity of the 5 NS3 polypeptide is disrupted. Methods of measuring protease activity are discussed further below.

By "fragment" is intended a polypeptide consisting of only a part of the intact full-length polypeptide sequence and structure. The fragment can include a C-terminal deletion and/or an N-terminal deletion of the native polypeptide. An 10 "immunogenic fragment" of a particular HCV protein will generally include at least about 5-10 contiguous amino acid residues of the full-length molecule, preferably at least about 15-25 contiguous amino acid residues of the full-length molecule, and most preferably at least about 20-50 or more contiguous amino acid residues of the full-length molecule, that define an epitope, or any integer between 5 amino acids and 15 the full-length sequence, provided that the fragment in question retains immunogenic activity, as measured by the assays described herein.

The term "epitope" as used herein refers to a sequence of at least about 3 to 5, preferably about 5 to 10 or 15, and not more than about 1,000 amino acids (or any integer therebetween), which define a sequence that by itself or as part of a larger 20 sequence, binds to an antibody generated in response to such sequence. There is no critical upper limit to the length of the fragment, which may comprise nearly the full-length of the protein sequence, or even a fusion protein comprising two or more epitopes from the HCV polyprotein. An epitope for use in the subject invention is not limited to a polypeptide having the exact sequence of the portion of the parent protein 25 from which it is derived. Indeed, viral genomes are in a state of constant flux and contain several variable domains which exhibit relatively high degrees of variability between isolates. Thus the term "epitope" encompasses sequences identical to the native sequence, as well as modifications to the native sequence, such as deletions, additions and substitutions (generally conservative in nature).

30 Regions of a given polypeptide that include an epitope can be identified using any number of epitope mapping techniques, well known in the art. See, e.g., *Epitope*

5 *Mapping Protocols* in Methods in Molecular Biology, Vol. 66 (Glenn E. Morris, Ed., 1996) Humana Press, Totowa, New Jersey. For example, linear epitopes may be determined by e.g., concurrently synthesizing large numbers of peptides on solid supports, the peptides corresponding to portions of the protein molecule, and reacting the peptides with antibodies while the peptides are still attached to the supports. Such techniques are known in the art and described in, e.g., U.S. Patent No. 4,708,871; Geysen et al. (1984) *Proc. Natl. Acad. Sci. USA* 81:3998-4002; Geysen et al. (1986) *Molec. Immunol.* 23:709-715. Similarly, conformational epitopes are readily identified by determining spatial conformation of amino acids such as by, e.g., x-ray 10 crystallography and 2-dimensional nuclear magnetic resonance. See, e.g., *Epitope Mapping Protocols*, *supra*. Antigenic regions of proteins can also be identified using standard antigenicity and hydropathy plots, such as those calculated using, e.g., the Omiga version 1.0 software program available from the Oxford Molecular Group. This computer program employs the Hopp/Woods method, Hopp et al., *Proc. Natl. Acad. Sci USA* (1981) 78:3824-3828 for determining antigenicity profiles, and the Kyte-Doolittle technique, Kyte et al., *J. Mol. Biol.* (1982) 157:105-132 for hydropathy 15 plots.

20 For a description of various HCV epitopes, see, e.g., Chien et al., *Proc. Natl. Acad. Sci. USA* (1992) 89:10011-10015; Chien et al., *J. Gastroent. Hepatol.* (1993) 8:S33-39; Chien et al., International Publication No. WO 93/00365; Chien, D.Y., International Publication No. WO 94/01778; and U.S. Patent Nos. 6,280,927 and 6,150,087.

25 As used herein, the term “conformational epitope” refers to a portion of a full-length protein, or an analog or mutein thereof, having structural features native to the amino acid sequence encoding the epitope within the full-length natural protein. Native structural features include, but are not limited to, glycosylation and three dimensional structure. Preferably, a conformational epitope is produced recombinantly and is expressed in a cell from which it is extractable under conditions which preserve its desired structural features, e.g. without denaturation of the epitope. 30 Such cells include bacteria, yeast, insect, and mammalian cells. Expression and isolation of recombinant conformational epitopes from the HCV polyprotein are

described in e.g., International Publication Nos. WO 96/04301, WO 94/01778, WO 95/33053, WO 92/08734.

As used herein the term “T-cell epitope” refers to a feature of a peptide structure which is capable of inducing T-cell immunity towards the peptide structure or an associated hapten. T-cell epitopes generally comprise linear peptide determinants that assume extended conformations within the peptide-binding cleft of MHC molecules, (Unanue et al., *Science* (1987) 236:551-557). Conversion of polypeptides to MHC class II-associated linear peptide determinants (generally between 5-14 amino acids in length) is termed “antigen processing” which is carried out by antigen presenting cells (APCs). More particularly, a T-cell epitope is defined by local features of a short peptide structure, such as primary amino acid sequence properties involving charge and hydrophobicity, and certain types of secondary structure, such as helicity, that do not depend on the folding of the entire polypeptide. Further, it is believed that short peptides capable of recognition by helper T-cells are generally amphipathic structures comprising a hydrophobic side (for interaction with the MHC molecule) and a hydrophilic side (for interacting with the T-cell receptor), (Margalit et al., *Computer Prediction of T-cell Epitopes, New Generation Vaccines* Marcel-Dekker, Inc, ed. G.C. Woodrow et al., (1990) pp. 109-116) and further that the amphipathic structures have an α -helical configuration (see, e.g., Spouge et al., *J. Immunol.* (1987) 138:204-212; Berkower et al., *J. Immunol.* (1986) 136:2498-2503).

Hence, segments of proteins that include T-cell epitopes can be readily predicted using numerous computer programs. (See e.g., Margalit et al., *Computer Prediction of T-cell Epitopes, New Generation Vaccines* Marcel-Dekker, Inc, ed. G.C. Woodrow et al., (1990) pp. 109-116). Such programs generally compare the amino acid sequence of a peptide to sequences known to induce a T-cell response, and search for patterns of amino acids which are believed to be required for a T-cell epitope.

An “immunological response” to an HCV antigen (including both polypeptide and polynucleotides encoding polypeptides that are expressed *in vivo*) or composition is the development in a subject of a humoral and/or a cellular immune response to molecules present in the composition of interest. For purposes of the present invention, a “humoral immune response” refers to an immune response mediated by

antibody molecules, while a "cellular immune response" is one mediated by T-lymphocytes and/or other white blood cells. One important aspect of cellular immunity involves an antigen-specific response by cytolytic T-cells ("CTLs"). CTLs have specificity for peptide antigens that are presented in association with proteins encoded by the major histocompatibility complex (MHC) and expressed on the surfaces of cells. CTLs help induce and promote the intracellular destruction of intracellular microbes, or the lysis of cells infected with such microbes. Another aspect of cellular immunity involves an antigen-specific response by helper T-cells. Helper T-cells act to help stimulate the function, and focus the activity of, nonspecific effector cells against cells displaying peptide antigens in association with MHC molecules on their surface. A "cellular immune response" also refers to the production of cytokines, chemokines and other such molecules produced by activated T-cells and/or other white blood cells, including those derived from CD4+ and CD8+ T-cells.

15 A composition or vaccine that elicits a cellular immune response may serve to sensitize a vertebrate subject by the presentation of antigen in association with MHC molecules at the cell surface. The cell-mediated immune response is directed at, or near, cells presenting antigen at their surface. In addition, antigen-specific T-lymphocytes can be generated to allow for the future protection of an immunized host.

20 The ability of a particular antigen to stimulate a cell-mediated immunological response may be determined by a number of assays, such as by lymphoproliferation (lymphocyte activation) assays, CTL cytotoxic cell assays, or by assaying for T-lymphocytes specific for the antigen in a sensitized subject. Such assays are well known in the art. See, e.g., Erickson et al., *J. Immunol.* (1993) 151:4189-4199; Doe et al., *Eur. J. Immunol.* (1994) 24:2369-2376; and the examples below.

25 Thus, an immunological response as used herein may be one which stimulates the production of CTLs, and/or the production or activation of helper T- cells. The antigen of interest may also elicit an antibody-mediated immune response. Hence, an immunological response may include one or more of the following effects: the production of antibodies by B-cells; and/or the activation of suppressor T-cells and/or

γδ T-cells directed specifically to an antigen or antigens present in the composition or vaccine of interest. These responses may serve to neutralize infectivity, and/or mediate antibody-complement, or antibody dependent cell cytotoxicity (ADCC) to provide protection or alleviation of symptoms to an immunized host. Such responses 5 can be determined using standard immunoassays and neutralization assays, well known in the art.

By "equivalent antigenic determinant" is meant an antigenic determinant from different sub-species or strains of HCV, such as from strains 1, 2, 3, etc., of HCV which antigenic determinants are not necessarily identical due to sequence variation, 10 but which occur in equivalent positions in the HCV sequence in question. In general the amino acid sequences of equivalent antigenic determinants will have a high degree of sequence homology, e.g., amino acid sequence homology of more than 30%, usually more than 40%, such as more than 60%, and even more than 80-90% homology, when the two sequences are aligned.

15 A "coding sequence" or a sequence which "encodes" a selected polypeptide, is a nucleic acid molecule which is transcribed (in the case of DNA) and translated (in the case of mRNA) into a polypeptide *in vitro* or *in vivo* when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon 20 at the 3' (carboxy) terminus. A transcription termination sequence may be located 3' to the coding sequence.

25 A "nucleic acid" molecule or "polynucleotide" can include both double- and single-stranded sequences and refers to, but is not limited to, cDNA from viral, procaryotic or eucaryotic mRNA, genomic DNA sequences from viral (e.g. DNA viruses and retroviruses) or procaryotic DNA, and especially synthetic DNA sequences. The term also captures sequences that include any of the known base analogs of DNA and RNA.

An "HCV polynucleotide" is a polynucleotide that encodes an HCV polypeptide, as defined above.

30 "Operably linked" refers to an arrangement of elements wherein the components so described are configured so as to perform their desired function. Thus,

a given promoter operably linked to a coding sequence is capable of effecting the expression of the coding sequence when the proper transcription factors, etc., are present. The promoter need not be contiguous with the coding sequence, so long as it functions to direct the expression thereof. Thus, for example, intervening untranslated 5 yet transcribed sequences can be present between the promoter sequence and the coding sequence, as can transcribed introns, and the promoter sequence can still be considered "operably linked" to the coding sequence.

"Recombinant" as used herein to describe a nucleic acid molecule means a polynucleotide of genomic, cDNA, viral, semisynthetic, or synthetic origin which, by 10 virtue of its origin or manipulation is not associated with all or a portion of the polynucleotide with which it is associated in nature. The term "recombinant" as used with respect to a protein or polypeptide means a polypeptide produced by expression of a recombinant polynucleotide. In general, the gene of interest is cloned and then expressed in transformed organisms, as described further below. The host organism 15 expresses the foreign gene to produce the protein under expression conditions.

A "control element" refers to a polynucleotide sequence which aids in the expression of a coding sequence to which it is linked. The term includes promoters, transcription termination sequences, upstream regulatory domains, polyadenylation signals, untranslated regions, including 5'-UTRs and 3'-UTRs and when appropriate, 20 leader sequences and enhancers, which collectively provide for the transcription and translation of a coding sequence in a host cell.

A "promoter" as used herein is a DNA regulatory region capable of binding RNA polymerase in a host cell and initiating transcription of a downstream (3' direction) coding sequence operably linked thereto. For purposes of the present 25 invention, a promoter sequence includes the minimum number of bases or elements necessary to initiate transcription of a gene of interest at levels detectable above background. Within the promoter sequence is a transcription initiation site, as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eucaryotic promoters will often, but not always, contain "TATA" boxes 30 and "CAT" boxes.

A control sequence “directs the transcription” of a coding sequence in a cell when RNA polymerase will bind the promoter sequence and transcribe the coding sequence into mRNA, which is then translated into the polypeptide encoded by the coding sequence.

5 “Expression cassette” or “expression construct” refers to an assembly which is capable of directing the expression of the sequence(s) or gene(s) of interest. The expression cassette includes control elements, as described above, such as a promoter which is operably linked to (so as to direct transcription of) the sequence(s) or gene(s) of interest, and often includes a polyadenylation sequence as well. Within certain
10 embodiments of the invention, the expression cassette described herein may be contained within a plasmid construct. In addition to the components of the expression cassette, the plasmid construct may also include, one or more selectable markers, a signal which allows the plasmid construct to exist as single-stranded DNA (e.g., a M13 origin of replication), at least one multiple cloning site, and a “mammalian”
15 origin of replication (e.g., a SV40 or adenovirus origin of replication).

“Transformation,” as used herein, refers to the insertion of an exogenous polynucleotide into a host cell, irrespective of the method used for insertion: for example, transformation by direct uptake, transfection, infection, and the like. For particular methods of transfection, see further below. The exogenous polynucleotide
20 may be maintained as a nonintegrated vector, for example, an episome, or alternatively, may be integrated into the host genome.

A “host cell” is a cell which has been transformed, or is capable of transformation, by an exogenous DNA sequence.

By “isolated” is meant, when referring to a polypeptide, that the indicated
25 molecule is separate and discrete from the whole organism with which the molecule is found in nature or is present in the substantial absence of other biological macromolecules of the same type. The term “isolated” with respect to a polynucleotide is a nucleic acid molecule devoid, in whole or part, of sequences normally associated with it in nature; or a sequence, as it exists in nature, but having heterologous sequences in
30 association therewith; or a molecule disassociated from the chromosome.

The term "purified" as used herein preferably means at least 75% by weight, more preferably at least 85% by weight, more preferably still at least 95% by weight, and most preferably at least 98% by weight, of biological macromolecules of the same type are present.

5 "Homology" refers to the percent identity between two polynucleotide or two polypeptide moieties. Two DNA, or two polypeptide sequences are "substantially homologous" to each other when the sequences exhibit at least about 50%, preferably at least about 75%, more preferably at least about 80%-85%, preferably at least about 90%, and most preferably at least about 95%-98%, or more, sequence identity over a
10 defined length of the molecules. As used herein, substantially homologous also refers to sequences showing complete identity to the specified DNA or polypeptide sequence.

In general, "identity" refers to an exact nucleotide-to-nucleotide or amino acid-to-amino acid correspondence of two polynucleotides or polypeptide sequences, 15 respectively. Percent identity can be determined by a direct comparison of the sequence information between two molecules by aligning the sequences, counting the exact number of matches between the two aligned sequences, dividing by the length of the shorter sequence, and multiplying the result by 100. Readily available computer programs can be used to aid in the analysis, such as ALIGN, Dayhoff, M.O. in *Atlas of*
20 *Protein Sequence and Structure* M.O. Dayhoff ed., 5 Suppl. 3:353-358, National biomedical Research Foundation, Washington, DC, which adapts the local homology algorithm of Smith and Waterman *Advances in Appl. Math.* 2:482-489, 1981 for peptide analysis. Programs for determining nucleotide sequence identity are available in the Wisconsin Sequence Analysis Package, Version 8 (available from Genetics
25 Computer Group, Madison, WI) for example, the BESTFIT, FASTA and GAP programs, which also rely on the Smith and Waterman algorithm. These programs are readily utilized with the default parameters recommended by the manufacturer and described in the Wisconsin Sequence Analysis Package referred to above. For example, percent identity of a particular nucleotide sequence to a reference sequence
30 can be determined using the homology algorithm of Smith and Waterman with a default scoring table and a gap penalty of six nucleotide positions.

Another method of establishing percent identity in the context of the present invention is to use the MPSRCH package of programs copyrighted by the University of Edinburgh, developed by John F. Collins and Shane S. Sturrok, and distributed by IntelliGenetics, Inc. (Mountain View, CA). From this suite of packages the Smith-Waterman algorithm can be employed where default parameters are used for the scoring table (for example, gap open penalty of 12, gap extension penalty of one, and a gap of six). From the data generated the "Match" value reflects "sequence identity." Other suitable programs for calculating the percent identity or similarity between sequences are generally known in the art, for example, another alignment program is BLAST, used with default parameters. For example, BLASTN and BLASTP can be used using the following default parameters: genetic code = standard; filter = none; strand = both; cutoff = 60; expect = 10; Matrix = BLOSUM62; Descriptions = 50 sequences; sort by = HIGH SCORE; Databases = non-redundant, GenBank + EMBL + DDBJ + PDB + GenBank CDS translations + Swiss protein + Spupdate + PIR.

Details of these programs can be found at the following internet address:
<http://www.ncbi.nlm.gov/cgi-bin/BLAST>.

Alternatively, homology can be determined by hybridization of polynucleotides under conditions which form stable duplexes between homologous regions, followed by digestion with single-stranded-specific nuclease(s), and size determination of the digested fragments. DNA sequences that are substantially homologous can be identified in a Southern hybridization experiment under, for example, stringent conditions, as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., Sambrook et al., *supra*; *DNA Cloning, supra*; *Nucleic Acid Hybridization, supra*.

By "nucleic acid immunization" is meant the introduction of a nucleic acid molecule encoding one or more selected antigens into a host cell, for the *in vivo* expression of the antigen or antigens. The nucleic acid molecule can be introduced directly into the recipient subject, such as by injection, inhalation, oral, intranasal and mucosal administration, or the like, or can be introduced *ex vivo*, into cells which have been removed from the host. In the latter case, the transformed cells are reintroduced

into the subject where an immune response can be mounted against the antigen encoded by the nucleic acid molecule.

As used herein, "treatment" refers to any of (i) the prevention of infection or reinfection, as in a traditional vaccine, (ii) the reduction or elimination of symptoms, 5 and (iii) the substantial or complete elimination of the pathogen in question.

Treatment may be effected prophylactically (prior to infection) or therapeutically (following infection).

By "vertebrate subject" is meant any member of the subphylum cordata, including, without limitation, humans and other primates, including non-human 10 primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats and guinea pigs; birds, including domestic, wild and game birds such as chickens, turkeys and other 15 gallinaceous birds, ducks, geese, and the like. The term does not denote a particular age. Thus, both adult and newborn individuals are intended to be covered. The invention described herein is intended for use in any of the above vertebrate species, 20 since the immune systems of all of these vertebrates operate similarly.

II. Modes of Carrying out the Invention

20 Before describing the present invention in detail, it is to be understood that this invention is not limited to particular formulations or process parameters as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting.

25 Although a number of compositions and methods similar or equivalent to those described herein can be used in the practice of the present invention, the preferred materials and methods are described herein.

It is a discovery of the present invention that fusion proteins, combinations of the individual components of these fusions, and polynucleotides encoding the same, 30 comprising an NS3, an NS4, and an NS5a polypeptide with or without a core polypeptide, or an NS3, an NS4, an NS5a, and an NS5b polypeptide, with or without a

core polypeptide, of an HCV virus can be used to activate HCV-specific T cells, i.e., T cells which recognize epitopes of these polypeptides.

The present invention also pertains to compositions comprising HCV nonstructural fusion proteins and HCV E1E2 complexes, as well as compositions 5 comprising polynucleotides encoding the same or combinations of polypeptides and polynucleotides.

The proteins, polynucleotides, compositions and combinations of the present invention can be used to stimulate a cellular immune response, such as to activate HCV-specific T cells, i.e., T cells which recognize epitopes of these polypeptides.

10 Activation of HCV-specific T cells provides both *in vitro* and *in vivo* model systems for the development of HCV vaccines, particularly for identifying HCV polypeptide epitopes associated with a response. The compositions can also be used to generate an immune response against HCV in a mammal, particularly a CTL response for either therapeutic or prophylactic purposes.

15

Fusion Proteins

The genomes of HCV strains contain a single open reading frame of approximately 9,000 to 12,000 nucleotides, which is transcribed into a polyprotein. As shown in Figure 1 and the table below, an HCV polyprotein, upon cleavage, 20 produces at least ten distinct products, in the order of NH₂-Core-E1-E2-p7-NS2-NS3-NS4a-NS4b-NS5a-NS5b-COOH. The core polypeptide occurs at positions 1-191, numbered relative to HCV-1 (see, Choo et al. (1991) *Proc. Natl. Acad. Sci. USA* **88**:2451-2455, for the HCV-1 genome). This polypeptide is further processed to produce an HCV polypeptide with approximately amino acids 1-173. The envelope 25 polypeptides, E1 and E2, occur at about positions 192-383 and 384-746, respectively. The P7 domain is found at about positions 747-809. NS2 is an integral membrane protein with proteolytic activity and is found at about positions 810-1026 of the polyprotein. NS2, in combination with NS3, (found at about positions 1027-1657), cleaves the NS2-NS3 sissle bond which in turn generates the NS3 N-terminus and 30 releases a large polyprotein that includes both serine protease and RNA helicase activities. The NS3 protease, found at about positions 1027-1207, serves to process

the remaining polyprotein. The helicase activity is found at about positions 1193-1657. NS3 liberates an NS3 cofactor (NS4a, found about positions 1658-1711), two proteins (NS4b found at about positions 1712-1972, and NS5a found at about positions 1973-2420), and an RNA-dependent RNA polymerase (NS5b found at about 5 positions 2421-3011). Completion of polyprotein maturation is initiated by autocatalytic cleavage at the NS3-Ns4a junction, catalyzed by the NS3 serine protease.

	Domain	Approximate Boundaries*
10	C (core)	1-191
	E1	192-383
	E2	384-746
	P7	747-809
	NS2	810-1026
	NS3	1027-1657
	NS4a	1658-1711
	NS4b	1712-1972
	NS5a	1973-2420
	NS5b	2421-3011

20 *Numbered relative to HCV-1. See, Choo et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:2451-2455.

25 Fusion proteins for use in the compositions and methods, and polynucleotides encoding therefor, include or encode an NS3 polypeptide, an NS4 (NS4a and/or NS4b) polypeptide, an NS5a polypeptide and, optionally, an NS5b polypeptide. The fusion proteins may or may not include all or part of the core region. In certain embodiments, none of the core region is present in the compositions. The nonstructural regions need not be in the order in which they naturally occur in the native HCV polyprotein. Thus, for example, the NS5b polypeptide may be at the N- 30 and/or C-terminus of the fusion or may be found internally. These polypeptides may

be derived from the same HCV isolate, or from different strains and isolates including isolates having any of the various HCV genotypes, to provide increased protection against a broad range of HCV genotypes. Additionally, polypeptides can be selected based on the particular viral clades endemic in specific geographic 5 regions where vaccine compositions containing the fusions will be used. It is readily apparent that the subject fusions provide an effective means of treating HCV infection in a wide variety of contexts.

In one embodiment, the fusion protein of the present invention includes an NS3 polypeptide that has been modified to inhibit protease activity, such that further 10 cleavage of the fusion is inhibited. The NS3 polypeptide can be modified by deletion of all or a portion of the NS3 protease domain. Alternatively, proteolytic activity can be inhibited by substitutions of amino acids within active regions of the protease domain. Finally, additions of amino acids to active regions of the domain, such that the catalytic site is modified, will also serve to inhibit proteolytic activity.

15 As explained above, the protease activity is found at about amino acid positions 1027-1207, numbered relative to the full-length HCV-1 polyprotein (see, Choo et al., *Proc. Natl. Acad. Sci. USA* (1991) 88:2451-2455), positions 2-182 of Figure 3. The structure of the NS3 protease and active site are known. See, e.g., De Francesco et al., *Antivir. Ther.* (1998) 3:99-109; Koch et al., *Biochemistry* (2001) 40:631-640. Thus, deletions or modifications to the native sequence will typically occur at or near the active site of the molecule. Particularly, it is desirable to modify or make deletions to one or more amino acids occurring at positions 1- or 2-182, preferably 1- or 2-170, or 1- or 2-155 of Figure 3. Preferred modifications are to the catalytic triad at the active site of the protease, i.e., H, D or S residues, in order to 20 inactivate the protease. These residues occur at positions 1083, 1105 and 1165, respectively, numbered relative to the full-length HCV polyprotein (positions 58, 80 and 140, respectively, of Figure 3). Such modifications will suppress proteolytic 25 cleavage while maintaining T-cell epitopes.

One of skill in the art can readily determine portions of the NS3 protease to delete in 30 order to disrupt activity. The presence or absence of activity can be determined using methods known to those of skill in the art.

For example, protease activity or lack thereof may be determined using assays well known in the art. See, e.g., Takeshita et al., *Anal. Biochem.* (1997) 247:242-246; Kakiuchi et al., *J. Biochem.* (1997) 122:749-755; Sali et al., *Biochemistry* (1998) 37:3392-3401; Cho et al., *J. Virol. Meth.* (1998) 72:109-115; Cerretani et al., 5 *Anal. Biochem.* (1999) 266:192-197; Zhang et al., *Anal. Biochem.* (1999) 270:268-275; Kakiuchi et al., *J. Virol. Meth.* (1999) 80:77-84; Fowler et al., *J. Biomol. Screen.* (2000) 5:153-158; and Kim et al., *Anal. Biochem.* (2000) 284:42-48.

The NS3, NS4, NS5a, and NS5b polypeptides present in the various fusions described above can either be full-length polypeptides or portions of NS3, NS4 10 (NS4a and/or NS4b), NS5a, and NS5b polypeptides. The portions of NS3, NS4, NS5a, and NS5b polypeptides making up the fusion protein preferably comprise at least one epitope, which is recognized by a T cell receptor on an activated T cell, such as 2152-HEYPVGSQ-2160 (SEQ ID NO:1) and/or 2224- 15 AELIEANLLWRQEMG-2238 (SEQ ID NO:2). Epitopes of NS3, NS4 (NS4a and NS4b), NS5a, NS5b, NS3NS4NS5a, and NS3NS4NS5aNS5b can be identified by several methods. For example, NS3, NS4, NS5a, NS5b polypeptides or fusion 20 proteins comprising any combination of the above, can be isolated, for example, by immunoaffinity purification using a monoclonal antibody for the polypeptide or protein. The isolated protein sequence can then be screened by preparing a series of short peptides by proteolytic cleavage of the purified protein, which together span the entire protein sequence. By starting with, for example, 100-mer polypeptides, each 25 polypeptide can be tested for the presence of epitopes recognized by a T-cell receptor on an HCV-activated T cell, progressively smaller and overlapping fragments can then be tested from an identified 100-mer to map the epitope of interest.

Epitopes recognized by a T-cell receptor on an HCV-activated T cell can be 30 identified by, for example, ⁵¹Cr release assay or by lymphoproliferation assay (see the examples). In a ⁵¹Cr release assay, target cells can be constructed that display the epitope of interest by cloning a polynucleotide encoding the epitope into an expression vector and transforming the expression vector into the target cells. HCV-specific CD8⁺ T cells will lyse target cells displaying, for example, an NS3, NS4, NS5a, NS5b, NS3NS4NS5a, or NS3NS4NS5aNS5b epitope and will not lyse cells

that do not display such an epitope. In a lymphoproliferation assay, HCV-activated CD4⁺ T cells will proliferate when cultured with, for example, an NS3, NS4, NS5a, NS5b, NS3NS4NS5a, or NS3NS4NS5aNS5b epitopic peptide, but not in the absence of an HCV epitopic peptide.

5 NS3, NS4, NS5a, and NS5b polypeptides can occur in any order in the fusion protein. If desired, at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more of one or more of the polypeptides may occur in the fusion protein. Multiple viral strains of HCV occur, and NS3, NS4, NS5a, and NS5b polypeptides of any of these strains can be used in a fusion protein. A representative fusion protein for use in the present invention is
10 shown in Figures 5A-5J. The depicted sequence includes amino acids 1242-3011 of the HCV polyprotein (representing polypeptides from NS3, NS4, NS5a and NS5b) with amino acids 1-121 of the HCV polyprotein (representing a polypeptide from the core region) fused to the C-terminus of NS5b. This numbering is relative to the HCV-1 polyprotein.

15 Nucleic acid and amino acid sequences of a number of HCV strains and isolates, including nucleic acid and amino acid sequences of NS3, NS4, NS5a, NS5b genes and polypeptides have been determined. For example, isolate HCV J1.1 is described in Kubo *et al.* (1989) Japan. Nucl. Acids Res. 17:10367-10372; Takeuchi *et al.* (1990) Gene 91:287-291; Takeuchi *et al.* (1990) J. Gen. Virol. 71:3027-3033; 20 and Takeuchi *et al.* (1990) Nucl. Acids Res. 18:4626. The complete coding sequences of two independent isolates, HCV-J and BK, are described by Kato *et al.*, (1990) Proc. Natl. Acad. Sci. USA 87:9524-9528 and Takamizawa *et al.*, (1991) J. Virol. 65:1105-1113 respectively.

25 Publications that describe HCV-1 isolates include Choo *et al.* (1990) Brit. Med. Bull. 46:423-441; Choo *et al.* (1991) Proc. Natl. Acad. Sci. USA 88:2451-2455 and Han *et al.* (1991) Proc. Natl. Acad. Sci. USA 88:1711-1715. HCV isolates HC-J1 and HC-J4 are described in Okamoto *et al.* (1991) Japan J. Exp. Med. 60:167-177. HCV isolates HCT 18~, HCT 23, Th, HCT 27, EC1 and EC10 are described in Weiner *et al.* (1991) Virol. 180:842-848. HCV isolates Pt-1, HCV-K1 30 and HCV-K2 are described in Enomoto *et al.* (1990) Biochem. Biophys. Res.

Commun. 170:1021-1025. HCV isolates A, C, D & E are described in Tsukiyama-Kohara *et al.* (1991) *Virus Genes* 5:243-254.

Each of the NS3, NS4, NS5a, and NS5b components of a fusion protein can be obtained from the same HCV strain or isolate or from different HCV strains or isolates. Fusion proteins comprising HCV polypeptides from, for example, the NS3 polypeptide can be derived from a first strain of HCV, and the NS4, and NS5a polypeptides can be derived from a second strain of HCV. Alternatively, the NS4 polypeptide can be derived from a first strain of HCV, and the NS3 and NS5a polypeptides can be derived from a second strain of HCV. Optionally, the NS5a polypeptide can be derived from a first strain of HCV, and the NS3 and NS4 polypeptides can be derived from a second strain of HCV. NS3, NS4 and NS5a polypeptides that are each derived from different HCV strains can also be used in an HCV fusion protein. Similarly, in a fusion protein comprising NS5b, at least one of the NS3, NS4, NS5a, and NS5b polypeptides can be derived from a different HCV strain than the other polypeptides. Optionally, NS3, NS4, NS5a, and NS5b polypeptides that are each derived from different HCV strains can also be used in an NS3NS4NS5aNS5b fusion protein.

In addition to NS3, NS4a, NS4b, NS5a and NS5b, the fusion proteins can contain other polypeptides derived from the HCV polyprotein. For example, it may be desirable to include polypeptides derived from the core region of the HCV polyprotein. This region occurs at amino acid positions 1-191 of the HCV polyprotein, numbered relative to HCV-1. Either the full-length protein, fragments thereof, such as amino acids 1-150, e.g., amino acids 1-130, 1-120, for example, amino acids 1-121, 1-122, 1-123, etc., or smaller fragments containing epitopes of the full-length protein may be used in the subject fusions, such as those epitopes found between amino acids 10-53, amino acids 10-45, amino acids 67-88, amino acids 120-130, or any of the core epitopes identified in, e.g., Houghton *et al.*, U.S. Patent No. 5,350,671; Chien *et al.*, *Proc. Natl. Acad. Sci. USA* (1992) 89:10011-10015; Chien *et al.*, *J. Gastroent. Hepatol.* (1993) 8:S33-39; Chien *et al.*, International Publication No. WO 93/00365; Chien, D.Y., International Publication No. WO 94/01778; and U.S. Patent Nos. 6,280,927 and 6,150,087. Moreover, a

protein resulting from a frameshift in the core region of the polyprotein, such as described in International Publication No. WO 99/63941, may be used. The fusions may also contain polynucleotides encoding E1E2 polypeptides, as described further below.

5 Preferably, the above-described fusion proteins, as well as the individual components of these proteins, are produced recombinantly. A polynucleotide encoding these proteins can be introduced into an expression vector which can be expressed in a suitable expression system. A variety of bacterial, yeast, mammalian and insect expression systems are available in the art and any such expression system 10 can be used. Optionally, a polynucleotide encoding these proteins can be translated in a cell-free translation system. Such methods are well known in the art. The proteins also can be constructed by solid phase protein synthesis.

15 If desired, the fusion proteins, or the individual components of these proteins, also can contain other non-HCV amino acid sequences, such as amino acid linkers or signal sequences, as well as ligands useful in protein purification, such as glutathione-S-transferase and staphylococcal protein A.

E1E2 Polypeptides

20 As explained above, the compositions of the present invention may also include E1 and E2 polypeptides, complexes of these polypeptides or polynucleotides encoding the same. The E1 and E2 polypeptides and complexes thereof can be provided independent of the nonstructural fusion protein or can be incorporated into the same fusion. Moreover, E1E2 complexes can be provided as proteins, or as 25 polynucleotides encoding the same.

25 In this regard, E1, E2 and p7 are known to contain human T-cell epitopes (both CD4+ and CD8+) and including one or more of these epitopes serves to increase vaccine efficacy as well as to increase protective levels against multiple HCV genotypes. Moreover, multiple copies of specific, conserved T-cell epitopes can also be used in E1E2 complexes, such as a composite of epitopes from different 30 genotypes.

As explained above, the E1 and E2 polypeptides that make up the E1E2 complexes can be associated either through non-covalent or covalent interactions. Such complexes may be made up of immunogenic fragments of E1 and E2 which comprise epitopes. For example, fragments of E1 polypeptides can comprise from 5 about 5 to nearly the full-length of the molecule, such as 6, 10, 25, 50, 75, 100, 125, 150, 175, 185 or more amino acids of an E1 polypeptide, or any integer between the stated numbers. Similarly, fragments of E2 polypeptides can comprise 6, 10, 25, 50, 75, 100, 150, 200, 250, 300, or 350 amino acids of an E2 polypeptide, or any integer between the stated numbers. The E1 and E2 polypeptides may be from the same or 10 different HCV strains. For example, epitopes derived from, e.g., the hypervariable region of E2, such as a region spanning amino acids 384-410 or 390-410, can be included in the E2 polypeptide. A particularly effective E2 epitope to incorporate into the E2 sequence or E1E2 complexes is one which includes a consensus sequence derived from this region, such as the consensus sequence Gly-Ser-Ala-Ala-Arg-Thr- 15 Thr-Ser-Gly-Phe-Val-Ser-Leu-Phe-Ala-Pro-Gly-Ala-Lys-Gln-Asn (SEQ ID NO:5), which represents a consensus sequence for amino acids 390-410 of the HCV type 1 genome. Additional epitopes of E1 and E2 are known and described in, e.g., Chien et al., International Publication No. WO 93/00365.

Moreover, the E1 and E2 polypeptides may lack all or a portion of the 20 membrane spanning domain. The membrane anchor sequence functions to associate the polypeptide to the endoplasmic reticulum. Normally, such polypeptides are capable of secretion into growth medium in which an organism expressing the protein is cultured. However, as described in International Publication No. WO 98/50556, such polypeptides may also be recovered intracellularly. Secretion into 25 growth medium is readily determined using a number of detection techniques, including, e.g., polyacrylamide gel electrophoresis and the like, and immunological techniques such as immunoprecipitation assays as described in, e.g., International Publication No. WO 96/04301, published February 15, 1996. With E1, generally polypeptides terminating with about amino acid position 370 and higher (based on 30 the numbering of HCV1 E1) will be retained by the ER and hence not secreted into growth media. With E2, polypeptides terminating with about amino acid position

731 and higher (also based on the numbering of the HCV1 E2 sequence) will be retained by the ER and not secreted. (See, e.g., International Publication No. WO 96/04301, published February 15, 1996). It should be noted that these amino acid positions are not absolute and may vary to some degree. Thus, the present invention 5 contemplates the use of E1 and E2 polypeptides which retain the transmembrane binding domain, as well as polypeptides which lack all or a portion of the transmembrane binding domain, including E1 polypeptides terminating at about amino acids 369 and lower, and E2 polypeptides, terminating at about amino acids 730 and lower, are intended to be captured by the present invention. Furthermore, 10 the C-terminal truncation can extend beyond the transmembrane spanning domain towards the N-terminus. Thus, for example, E1 truncations occurring at positions lower than, e.g., 360 and E2 truncations occurring at positions lower than, e.g., 715, are also encompassed by the present invention. All that is necessary is that the truncated E1 and E2 polypeptides remain functional for their intended purpose.

15 However, particularly preferred truncated E1 constructs are those that do not extend beyond about amino acid 300. Most preferred are those terminating at position 360. Preferred truncated E2 constructs are those with C-terminal truncations that do not extend beyond about amino acid position 715. Particularly preferred E2 truncations are those molecules truncated after any of amino acids 715-730, such as 725. If 20 truncated molecules are used, it is preferable to use E1 and E2 molecules that are both truncated.

E2 exists as multiple species (Spaete et al., *Virol.* (1992) 188:819-830; Selby et al., *J. Virol.* (1996) 70:5177-5182; Grakoui et al., *J. Virol.* (1993) 67:1385-1395; Tomei et al., *J. Virol.* (1993) 67:4017-4026) and clipping and proteolysis may occur 25 at the N- and C-termini of the E1 and E2 polypeptides. Thus, an E2 polypeptide for use herein may comprise at least amino acids 405-661, e.g., 400, 401, 402... to 661, such as 384-661, 384-715, 384-746, 384-749 or 384-809, or 384 to any C-terminus between 661-809, of an HCV polyprotein, numbered relative to the full-length HCV-1 polyprotein. Similarly, preferable E1 polypeptides for use herein can comprise 30 amino acids 192-326, 192-330, 192-333, 192-360, 192-363, 192-383, or 192 to any C-terminus between 326-383, of an HCV polyprotein.

The E1 and E2 polypeptides and complexes thereof may also be present as asialoglycoproteins. Such asialoglycoproteins are produced by methods known in the art, such as by using cells in which terminal glycosylation is blocked. When these proteins are expressed in such cells and isolated by GNA lectin affinity

5 chromatography, the E1 and E2 proteins aggregate spontaneously. Detailed methods for producing these E1E2 aggregates are described in, e.g., U.S. Patent No. 6,074,852. For example, E1E2 complexes are readily produced recombinantly, either as fusion proteins or by e.g., co-transfected host cells with constructs encoding for the E1 and E2 polypeptides of interest. Co-transfection can be accomplished either

10 in *trans* or *cis*, i.e., by using separate vectors or by using a single vector which bears both of the E1 and E2 genes. If done using a single vector, both genes can be driven by a single set of control elements or, alternatively, the genes can be present on the vector in individual expression cassettes, driven by individual control elements. Following expression, the E1 and E2 proteins will spontaneously associate.

15 Alternatively, the complexes can be formed by mixing the individual proteins together which have been produced separately, either in purified or semi-purified form, or even by mixing culture media in which host cells expressing the proteins, have been cultured, if the proteins are secreted. Finally, the E1E2 complexes of the present invention may be expressed as a fusion protein wherein the desired portion of

20 E1 is fused to the desired portion of E2.

Moreover, the E1E2 complexes may be present as a heterogeneous mixture of molecules, due to clipping and proteolytic cleavage, as described above. Thus, a composition including E1E2 complexes may include multiple species of E1E2, such as E1E2 terminating at amino acid 746 (E1E2₇₄₆), E1E2 terminating at amino acid 809 (E1E2₈₀₉), or any of the other various E1 and E2 molecules described above, such as E2 molecules with N-terminal truncations of from 1-20 amino acids, such as E2 species beginning at amino acid 387, amino acid 402, amino acid 403, etc.

30 E1E2 complexes are readily produced recombinantly, either as fusion proteins or by e.g., co-transfected host cells with constructs encoding for the E1 and E2 polypeptides of interest. Co-transfection can be accomplished either in *trans* or *cis*, i.e., by using separate vectors or by using a single vector which bears both of the E1

and E2 genes. If done using a single vector, both genes can be driven by a single set of control elements or, alternatively, the genes can be present on the vector in individual expression cassettes, driven by individual control elements. Following expression, the E1 and E2 proteins will spontaneously associate. Alternatively, the 5 complexes can be formed by mixing the individual proteins together which have been produced separately, either in purified or semi-purified form, or even by mixing culture media in which host cells expressing the proteins, have been cultured, if the proteins are secreted. Finally, the E1E2 complexes of the present invention may be expressed as a fusion protein wherein the desired portion of E1 is fused to the desired 10 portion of E2.

Methods for producing E1E2 complexes from full-length, truncated E1 and E2 proteins which are secreted into media, as well as intracellularly produced truncated proteins, are known in the art. For example, such complexes may be produced recombinantly, as described in U.S. Patent No. 6,121,020; Ralston et al., *J. Virol.* (1993) 67:6753-6761, Grakoui et al., *J. Virol.* (1993) 67:1385-1395; and 15 Lanford et al., *Virology* (1993) 197:225-235.

Polynucleotides Encoding the Fusion Proteins and E1E2 Complexes

Polynucleotides contain less than an entire HCV genome and can be RNA or 20 single- or double-stranded DNA. Preferably, the polynucleotides are isolated free of other components, such as proteins and lipids. The polynucleotides encode the fusion proteins, E1 and E2 polypeptides and complexes thereof, described above, and thus comprise coding sequences thereof. Polynucleotides of the invention can also comprise other non-HCV nucleotide sequences, such as sequences coding for linkers, 25 signal sequences, or ligands useful in protein purification such as glutathione-S-transferase and staphylococcal protein A.

Polynucleotides encoding the various HCV polypeptides can be isolated from a genomic library derived from nucleic acid sequences present in, for example, the plasma, serum, or liver homogenate of an HCV infected individual or can be 30 synthesized in the laboratory, for example, using an automatic synthesizer. An

amplification method such as PCR can be used to amplify polynucleotides from either HCV genomic DNA or cDNA encoding therefor.

Polynucleotides can comprise coding sequences for these polypeptides which occur naturally or can include artificial sequences which do not occur in nature.

5 These polynucleotides can be ligated to form a coding sequence for the fusion proteins and E1E2 complexes using standard molecular biology techniques. If desired, polynucleotides can be cloned into an expression vector and transformed into, for example, bacterial, yeast, insect, or mammalian cells so that the fusion proteins of the invention can be expressed in and isolated from a cell culture.

10 The expression constructs of the present invention, including the desired fusion, or individual expression constructs comprising the individual components of these fusions, may be used for nucleic acid immunization, to stimulate an immunological response, such as a cellular immune response, using standard gene delivery protocols. Methods for gene delivery are known in the art. See, e.g., U.S.

15 Patent Nos. 5,399,346, 5,580,859, 5,589,466. Genes can be delivered either directly to the vertebrate subject or, alternatively, delivered *ex vivo*, to cells derived from the subject and the cells reimplanted in the subject. For example, the constructs can be delivered as plasmid DNA, e.g., contained within a plasmid, such as pBR322, pUC, or ColE1

20 Additionally, the expression constructs can be packaged in liposomes prior to delivery to the cells. Lipid encapsulation is generally accomplished using liposomes which are able to stably bind or entrap and retain nucleic acid. The ratio of condensed DNA to lipid preparation can vary but will generally be around 1:1 (mg DNA:micromoles lipid), or more of lipid. For a review of the use of liposomes as carriers for delivery of nucleic acids, see, Hug and Sleight, *Biochim. Biophys. Acta*. (1991) 1097:1-17; Straubinger et al., in *Methods of Enzymology* (1983), Vol. 101, pp. 512-527.

30 Liposomal preparations for use with the present invention include cationic (positively charged), anionic (negatively charged) and neutral preparations, with cationic liposomes particularly preferred. Cationic liposomes are readily available. For example, N[1-2,3-dioleyloxy)propyl]-N,N,N-triethylammonium (DOTMA)

liposomes are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, NY. (See, also, Felgner et al., *Proc. Natl. Acad. Sci. USA* (1987) 84:7413-7416). Other commercially available lipids include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer). Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g., Szoka et al., *Proc. Natl. Acad. Sci. USA* (1978) 75:4194-4198; PCT Publication No. WO 90/11092 for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes. The various liposome-nucleic acid complexes are prepared using methods known in the art. See, e.g., Straubinger et al., in METHODS OF IMMUNOLOGY (1983), Vol. 101, pp. 512-527; Szoka et al., *Proc. Natl. Acad. Sci. USA* (1978) 75:4194-4198; Papahadjopoulos et al., *Biochim. Biophys. Acta* (1975) 394:483; Wilson et al., *Cell* (1979) 17:77; Deamer and Bangham, *Biochim. Biophys. Acta* (1976) 443:629; Ostro et al., *Biochem. Biophys. Res. Commun.* (1977) 76:836; Fraley et al., *Proc. Natl. Acad. Sci. USA* (1979) 76:3348; Enoch and Strittmatter, *Proc. Natl. Acad. Sci. USA* (1979) 76:145; Fraley et al., *J. Biol. Chem.* (1980) 255:10431; Szoka and Papahadjopoulos, *Proc. Natl. Acad. Sci. USA* (1978) 75:145; and Schaefer-Ridder et al., *Science* (1982) 215:166.

The DNA can also be delivered in cochleate lipid compositions similar to those described by Papahadjopoulos et al., *Biochim. Biophys. Acta* (1975) 394:483-491. See, also, U.S. Patent Nos. 4,663,161 and 4,871,488.

A number of viral-based systems have been developed for gene transfer into mammalian cells. For example, retroviruses provide a convenient platform for gene delivery systems, such as murine sarcoma virus, mouse mammary tumor virus, Moloney murine leukemia virus, and Rous sarcoma virus. A selected gene can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to cells of the subject either *in vivo* or *ex vivo*. A number of retroviral systems have been described (U.S. Patent No. 5,219,740; Miller and Rosman, *BioTechniques* (1989) 7:980-990; Miller, A.D., *Human Gene Therapy* (1990) 1:5-14; Scarpa et al., *Virology* (1991) 180:849-852; Burns et al., *Proc. Natl. Acad. Sci. USA* (1993) 90:8033-8037; and Boris-Lawrie and Temin, *Cur. Opin. Genet. Develop.* (1993) 3:102-109. Briefly,

retroviral gene delivery vehicles of the present invention may be readily constructed from a wide variety of retroviruses, including for example, B, C, and D type retroviruses as well as spumaviruses and lentiviruses such as FIV, HIV, HIV-1, HIV-2 and SIV (see RNA Tumor Viruses, Second Edition, Cold Spring Harbor 5 Laboratory, 1985). Such retroviruses may be readily obtained from depositories or collections such as the American Type Culture Collection ("ATCC"; 10801 University Blvd., Manassas, VA 20110-2209), or isolated from known sources using commonly available techniques.

A number of adenovirus vectors have also been described, such as adenovirus 10 Type 2 and Type 5 vectors. Unlike retroviruses which integrate into the host genome, adenoviruses persist extrachromosomally thus minimizing the risks associated with insertional mutagenesis (Haj-Ahmad and Graham, *J. Virol.* (1986) 57:267-274; Bett et al., *J. Virol.* (1993) 67:5911-5921; Mittereder et al., *Human Gene Therapy* (1994) 5:717-729; Seth et al., *J. Virol.* (1994) 68:933-940; Barr et al., *Gene Therapy* (1994) 1:51-58; Berkner, K.L. *BioTechniques* (1988) 6:616-629; and Rich et al., *Human Gene Therapy* (1993) 4:461-476).

Molecular conjugate vectors, such as the adenovirus chimeric vectors described in Michael et al., *J. Biol. Chem.* (1993) 268:6866-6869 and Wagner et al., *Proc. Natl. Acad. Sci. USA* (1992) 89:6099-6103, can also be used for gene delivery.

20 Members of the Alphavirus genus, such as but not limited to vectors derived from the Sindbis and Semliki Forest viruses, VEE, will also find use as viral vectors for delivering the gene of interest. For a description of Sindbis-virus derived vectors useful for the practice of the instant methods, see, Dubensky et al., *J. Virol.* (1996) 70:508-519; and International Publication Nos. WO 95/07995, and WO 96/17072.

25 Other vectors can be used, including but not limited to simian virus 40 and cytomegalovirus. Bacterial vectors, such as *Salmonella* ssp. *Yersinia enterocolitica*, *Shigella* spp., *Vibrio cholerae*, *Mycobacterium* strain BCG, and *Listeria monocytogenes* can be used. Minichromosomes such as MC and MC1, bacteriophages, cosmids (plasmids into which phage lambda *cos* sites have been 30 inserted) and replicons (genetic elements that are capable of replication under their own control in a cell) can also be used.

The expression constructs may also be encapsulated, adsorbed to, or associated with, particulate carriers. Such carriers present multiple copies of a selected molecule to the immune system and promote trapping and retention of molecules in local lymph nodes. The particles can be phagocytosed by macrophages 5 and can enhance antigen presentation through cytokine release. Examples of particulate carriers include those derived from polymethyl methacrylate polymers, as well as microparticles derived from poly(lactides) and poly(lactide-co-glycolides), known as PLG. See, e.g., Jeffery et al., *Pharm. Res.* (1993) 10:362-368; and McGee et al., *J. Microencap.* (1996).

10 One preferred method for adsorbing macromolecules onto prepared microparticles is described in International Publication No. WO 00/050006. Briefly, microparticles are rehydrated and dispersed to an essentially monomeric suspension of microparticles using dialyzable anionic or cationic detergents. Useful detergents include, but are not limited to, any of the various N-methylglucamides (known as 15 MEGAs), such as heptanoyl-N-methylglucamide (MEGA-7), octanoyl-N-methylglucamide (MEGA-8), nonanoyl-N-methylglucamide (MEGA-9), and decanoyl-N-methyl-glucamide (MEGA-10); cholic acid; sodium cholate; deoxycholic acid; sodium deoxycholate; taurocholic acid; sodium taurocholate; taurodeoxycholic acid; sodium taurodeoxycholate; 3-[(3- 20 cholamidopropyl)dimethylammonio] -1-propane-sulfonate (CHAPS); 3-[(3-cholamidopropyl) dimethylammonio]-2-hydroxy-1-propane-sulfonate (CHAPSO); -dodecyl-N,N-dimethyl-3-ammonio-1-propane-sulfonate (ZWITTERGENT 3-12); N,N-bis-(3-D-gluconeamidopropyl)-deoxycholamide (DEOXY-BIGCHAP); -octylglucoside; sucrose monolaurate; glycocholic acid/sodium glycocholate; 25 laurosarcosine (sodium salt); glycodeoxycholic acid/sodium glycodeoxycholate; sodium dodceyl sulfate (SDS); 3-(trimethylsilyl)-1-propanesulfonic acid (DSS); cetrimide (CTAB, the principal component of which is hexadecyltrimethylammonium bromide); hexadecyltrimethylammonium bromide; dodecyltrimethylammonium bromide; hexadecyltrimethylammonium bromide; 30 tetradecyltrimethylammonium bromide; benzyl dimethyldodecylammonium bromide; benzyl dimethyl-hexadecylammonium chloride; and benzyl dimethyltetra-

decylammonium bromide. The above detergents are commercially available from e.g., Sigma Chemical Co., St. Louis, MO. Various cationic lipids known in the art can also be used as detergents. See Balasubramaniam et al., 1996, *Gene Ther.*, 3:163-72 and Gao, X., and L. Huang. 1995, *Gene Ther.*, 2:7110-722.

5 A wide variety of other methods can be used to deliver the expression constructs to cells. Such methods include DEAE dextran-mediated transfection, calcium phosphate precipitation, polylysine- or polyornithine-mediated transfection, or precipitation using other insoluble inorganic salts, such as strontium phosphate, aluminum silicates including bentonite and kaolin, chromic oxide, magnesium silicate, talc, and the like. Other useful methods of transfection include 10 electroporation, sonoporation, protoplast fusion, liposomes, peptoid delivery, or microinjection. See, e.g., Sambrook et al., *supra*, for a discussion of techniques for transforming cells of interest; and Felgner, P.L., *Advanced Drug Delivery Reviews* (1990) 5:163-187, for a review of delivery systems useful for gene transfer. Methods 15 of delivering DNA using electroporation are described in, e.g., U.S. Patent Nos. 6,132,419; 6,451,002, 6,418,341, 6233,483, U.S. Patent Publication No. 2002/0146831; and International Publication No. WO/0045823.

Moreover, the HCV polynucleotides can be adsorbed to, or entrapped within, 20 an ISCOM. Classic ISCOMs are formed by combination of cholesterol, saponin, phospholipid, and immunogens, such as viral envelope proteins. Generally, the HCV molecules (usually with a hydrophobic region) are solubilized in detergent and added to the reaction mixture, whereby ISCOMs are formed with the HCV molecule incorporated therein. ISCOM matrix compositions are formed identically, but 25 without viral proteins. Proteins with high positive charge may be electrostatically bound in the ISCOM particles, rather than through hydrophobic forces. For a more detailed general discussion of saponins and ISCOMs, and methods of formulating ISCOMs, see Barr et al. (1998) *Adv. Drug Delivery Reviews* 32:247-271 (1998); U.S. Patent Nos. 4,981,684, 5,178,860, 5,679,354 and 6,027,732; European Publ. Nos. EPA 109,942; 180,564 and 231,039; and Coulter et al. (1998) *Vaccine* 16:1243.

30 Additionally, biolistic delivery systems employing particulate carriers such as gold and tungsten, are especially useful for delivering the expression constructs of

the present invention. The particles are coated with the construct to be delivered and accelerated to high velocity, generally under a reduced atmosphere, using a gun powder discharge from a "gene gun." For a description of such techniques, and apparatuses useful therefore, see, e.g., U.S. Patent Nos. 4,945,050; 5,036,006; 5,100,792; 5,179,022; 5,371,015; and 5,478,744.

Compositions Comprising Fusion Proteins or Polynucleotides

The invention also provides compositions comprising the fusion proteins or polynucleotides, as well as compositions including the individual components of these fusion proteins or polynucleotides. Compositions of the invention preferably comprise a pharmaceutically acceptable carrier. The carrier should not itself induce the production of antibodies harmful to the host. Pharmaceutically acceptable carriers are well known to those in the art. Such carriers include, but are not limited to, large, slowly metabolized, macromolecules, such as proteins, polysaccharides such as latex functionalized sepharose, agarose, cellulose, cellulose beads and the like, polylactic acids, polyglycolic acids, polymeric amino acids such as polyglutamic acid, polylysine, and the like, amino acid copolymers, and inactive virus particles.

Pharmaceutically acceptable salts can also be used in compositions of the invention, for example, mineral salts such as hydrochlorides, hydrobromides, phosphates, or sulfates, as well as salts of organic acids such as acetates, propionates, malonates, or benzoates. Especially useful protein substrates are serum albumins, keyhole limpet hemocyanin, immunoglobulin molecules, thyroglobulin, ovalbumin, tetanus toxoid, and other proteins well known to those of skill in the art. Compositions of the invention can also contain liquids or excipients, such as water, saline, glycerol, dextrose, ethanol, or the like, singly or in combination, as well as substances such as wetting agents, emulsifying agents, or pH buffering agents. Liposomes can also be used as a carrier for a composition of the invention, such liposomes are described above.

If desired, co-stimulatory molecules which improve immunogen presentation to lymphocytes, such as B7-1 or B7-2, or cytokines such as GM-CSF, IL-2, and IL-

12, can be included in a composition of the invention. Optionally, adjuvants can also be included in a composition. Adjuvants which can be used include, but are not limited to: (1) aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, aluminum sulfate, etc.; (2) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59 (U.S. Patent No. 6,299,884; Chapter 10 in *Vaccine design: the subunit and adjuvant approach*, eds. Powell & Newman, Plenum Press 1995), containing 5% Squalene, 0.5% TWEEN 80TM, and 0.5% SPAN 85TM (optionally containing various amounts of 5 MTP-PE (see below), although not required) formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA), (b) SAF, containing 10% Squalane, 0.4% TWEEN 80TM, 5% pluronic-blocked 10 polymer L121, and thr-MDP either microfluidized into a submicron emulsion or 15 vortexed to generate a larger particle size emulsion, and (c) RIBITM adjuvant system (RAS), (Ribi Immunochem, Hamilton, MT) containing 2% Squalene, 0.2% TWEEN 80TM, and one or more bacterial cell wall components from the group consisting of 20 monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (DETOXTM); (3) saponin adjuvants, such as QS21 or STIMULONTM (Cambridge Bioscience, Worcester, MA) may be used or particles 25 generated therefrom such as ISCOMs (immunostimulating complexes), which ISCOMs may be devoid of additional detergent, see, e.g., International Publication No. WO 00/07621; (4) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (5) cytokines, such as interleukins (IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12 (International Publication No. WO 99/44636), etc.), interferons (e.g., 30 gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), etc.; (6) detoxified mutants of a bacterial ADP-ribosylating toxin such as a cholera toxin (CT), a pertussis toxin (PT), or an *E. coli* heat-labile toxin (LT), particularly LT-K63 (where lysine is substituted for the wild-type amino acid at position 63) LT-R72 (where arginine is substituted for the wild-type amino acid at position 72), CT-S109 (where serine is substituted for the wild-type amino acid at position 109), and PT-K9/G129 (where lysine is substituted for the wild-type amino

acid at position 9 and glycine substituted at position 129) (see, e.g., International Publication Nos. W093/13202 and W092/19265); (7) MPL or 3-O-deacylated MPL (3dMPL) (see, e.g., GB 2220221), EP-A-0689454, optionally in the substantial absence of alum when used with pneumococcal saccharides (see, e.g., International Publication No. WO 00/56358); (8) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions (see, e.g., EP-A-0835318, EP-A-0735898, EP-A-0761231; (9) oligonucleotides comprising CpG motifs (see, e.g., Roman et al. (1997) *Nat. Med.* 3:849-854; Weiner et al. (1997) *Proc. Natl. Acad. Sci. USA* 94:10833-10837; Davis et al. (1998) *J. Immunol.* 160:870-876; Chu et al. (1997) *J. Exp. Med.* 186:1623-1631; Lipford et al. (1997) *Eur. J. Immunol.* 27:2340-2344; Moldoveanu et al. (1988) *Vaccine* 16:1216-1224; Krieg et al. (1995) *Nature* 374:546-549; Klinman et al. (1996) *Proc. Natl. Acad. Sci. USA* 93:2879-2883; Ballas et al. (1996) *J. Immunol.* 157:1840-1845; Cowdery et al. (1996) *J. Immunol.* 156:4570-4575; Halpern et al. (1996) *Cell Immunol.* 167:72-78; Yamamoto et al. (1988) *Jpn. J. Cancer Res.* 79:866-873; Stacey et al. (1996) *J. Immunol.* 157:2116-2122; Messina et al. (1991) *J. Immunol.* 147:1759-1764; Yi et al. (1996) *J. Immunol.* 157:4918-4925; Yi et al. (1996) *J. Immunol.* 157:5394-5402; Yi et al. (1998) *J. Immunol.* 160:4755-4761; Yi et al. (1998) *J. Immunol.* 160:5898-5906; International Publication Nos. WO 96/02555, WO 98/16247, WO 98/18810, WO 98/40100, WO 98/55495, WO 98/37919 and WO 98/52581), such as those containing at least one dinucleotide, with cytosine optionally replaced with 5-methylcytosine; (10) a polyoxyethylene ether or a polyoxyethylene ester (see, e.g., International Publication No. WO 99/52549); (11) a polyoxyethylene sorbitan ester surfactant in combination with an octoxynol (see, e.g., International Publication No. WO 01/21207) or a polyoxyethylene alkyl ether or ester surfactant in combination with at least one additional non-ionic surfactant such as an octoxynol (see, e.g., International Publication No. WO 01/21152); (12) a saponin and an immunostimulatory oligonucleotide such as a CpG oligonucleotide (see, e.g., International Publication No. WO 00/62800); (13) an immunostimulant and a particle of metal salt (see, e.g., International Publication No. WO 00/23105); and (14) other substances that act as immunostimulating agents to enhance the effectiveness of the composition.

Muramyl peptides include, but are not limited to, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), -acetylmuramyl-L-alanyl-D-isoglutamyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE), etc.

5 Moreover, the HCV proteins can be adsorbed to, or entrapped within, an ISCOM, as described above. Additionally, ISCOMs with adsorbed HCV core proteins, either the entire core region or a fragment of HCV core protein, may be added to the formulations. Most preferably, the HCV core protein is a fragment comprising a polypeptide from the region spanning amino acid positions 121-135.

10 See, e.g., International Publication No. WO 01/37869A.

As explained above, the composition may also contain immunostimulatory molecules, either in addition to or in place of the antigen delivery system.

15 Immunostimulatory agents for use herein include, without limitation, monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (DetoxTM). MPL may be formulated into an emulsion to enhance its immunostimulatory affect. See, e.g., Ulrich et al., "MPL_r immunostimulatory adjuvant formulations." in Vaccine Adjuvants: Preparation Methods and Research Protocols (O'Hagan DT, ed.) Human Press Inc., NJ (2000) pp. 273-282. MPL has been shown to induce the synthesis and release of cytokines, 20 particularly IL-2 and IFN- γ . Other useful immunostimulatory molecules include LPS and immunostimulatory nucleic acid sequences (ISS), including but not limited to, unmethylated CpG motifs, such as CpG oligonucleotides.

25 Oligonucleotides containing unmethylated CpG motifs have been shown to induce activation of B cells, NK cells and antigen-presenting cells (APCs), such as monocytes and macrophages. See, e.g., U.S. Patent No. 6,207,646. Thus, adjuvants derived from the CpG family of molecules, CpG dinucleotides and synthetic oligonucleotides which comprise CpG motifs (see, e.g., Krieg et al. *Nature* (1995) 374:546 and Davis et al. *J. Immunol.* (1998) 160:870-876) such as any of the various immunostimulatory CpG oligonucleotides disclosed in U.S. Patent No. 6,207,646, 30 may be used in the subject methods and compositions. Such CpG oligonucleotides generally comprise at least 8 up to about 100 basepairs, preferably 8 to 40 basepairs,

more preferably 15-35 basepairs, preferably 15-25 basepairs, and any number of basepairs between these values. For example, oligonucleotides comprising the consensus CpG motif, represented by the formula 5'-X₁CGX₂-3', where X₁ and X₂ are nucleotides and C is unmethylated, will find use as immunostimulatory CpG molecules. Generally, X₁ is A, G or T, and X₂ is C or T. Other useful CpG molecules include those captured by the formula 5'-X₁X₂CGX₃X₄, where X₁ and X₂ are a sequence such as GpT, GpG, GpA, ApA, ApT, ApG, CpT, CpA, CpG, TpA, TpT or TpG, and X₃ and X₄ are TpT, CpT, ApT, ApG, CpG, TpC, ApC, CpC, TpA, ApA, GpT, CpA, or TpG, wherein "p" signifies a phosphate bond. Preferably, the oligonucleotides do not include a GCG sequence at or near the 5'- and/or 3' terminus. Additionally, the CpG is preferably flanked on its 5'-end with two purines (preferably a GpA dinucleotide) or with a purine and a pyrimidine (preferably, GpT), and flanked on its 3'-end with two pyrimidines, preferably a TpT or TpC dinucleotide. Thus, preferred molecules will comprise the sequence GACGTT, GACGTC, GTCGTT or GTCGCT, and these sequences will be flanked by several additional nucleotides. The nucleotides outside of this central core area appear to be extremely amendable to change.

Moreover, the CpG oligonucleotides for use herein may be double- or single-stranded. Double-stranded molecules are more stable *in vivo* while single-stranded molecules display enhanced immune activity. Additionally, the phosphate backbone may be modified, such as phosphorodithioate-modified, in order to enhance the immunostimulatory activity of the CpG molecule. As described in U.S. Patent No. 6,207,646, CpG molecules with phosphorothioate backbones preferentially activate B-cells, while those having phosphodiester backbones preferentially activate 25 monocytic (macrophages, dendritic cells and monocytes) and NK cells.

One exemplary CpG oligonucleotide for use in the present compositions has the sequence 5'-TCCATGACGTTCCCTGACGTT-3' (SEQ ID NO:6).

CpG molecules can readily be tested for their ability to stimulate an immune response using standard techniques, well known in the art. For example, the ability 30 of the molecule to stimulate a humoral and/or cellular immune response is readily determined using the immunoassays described above. Moreover, the antigen and

adjuvant compositions can be administered with and without the CpG molecule to determine whether an immune response is enhanced.

The HCV proteins may also be encapsulated, adsorbed to, or associated with, particulate carriers, as described above with reference to the HCV polynucleotides.

5 As explained above, examples of particulate carriers include those derived from polymethyl methacrylate polymers, as well as microparticles derived from poly(lactides) and poly(lactide-co-glycolides), known as PLG. See, e.g., Jeffery et al., *Pharm. Res.* (1993) 10:362-368; and McGee et al., *J. Microencap.* (1996). One preferred method for adsorbing macromolecules onto prepared microparticles is

10 described above and in International Publication No. WO 00/050006.

Methods of Producing HCV-Specific Antibodies

The HCV proteins can be used to produce HCV-specific polyclonal and monoclonal antibodies. HCV-specific polyclonal and monoclonal antibodies 15 specifically bind to HCV antigens. Polyclonal antibodies can be produced by administering the fusion protein to a mammal, such as a mouse, a rabbit, a goat, or a horse. Serum from the immunized animal is collected and the antibodies are purified from the plasma by, for example, precipitation with ammonium sulfate, followed by chromatography, preferably affinity chromatography. Techniques for producing and 20 processing polyclonal antisera are known in the art.

Monoclonal antibodies directed against HCV-specific epitopes present in the proteins can also be readily produced. Normal B cells from a mammal, such as a mouse, immunized with an HCV protein, can be fused with, for example, HAT-sensitive mouse myeloma cells to produce hybridomas. Hybridomas producing 25 HCV-specific antibodies can be identified using RIA or ELISA and isolated by cloning in semi-solid agar or by limiting dilution. Clones producing HCV-specific antibodies are isolated by another round of screening.

Antibodies, either monoclonal and polyclonal, which are directed against HCV epitopes, are particularly useful for detecting the presence of HCV or HCV 30 antigens in a sample, such as a serum sample from an HCV-infected human. An immunoassay for an HCV antigen may utilize one antibody or several antibodies. An

immunoassay for an HCV antigen may use, for example, a monoclonal antibody directed towards an HCV epitope, a combination of monoclonal antibodies directed towards epitopes of one HCV polypeptide, monoclonal antibodies directed towards epitopes of different HCV polypeptides, polyclonal antibodies directed towards the same HCV antigen, polyclonal antibodies directed towards different HCV antigens, or a combination of monoclonal and polyclonal antibodies. Immunoassay protocols may be based, for example, upon competition, direct reaction, or sandwich type assays using, for example, labeled antibody. The labels may be, for example, fluorescent, chemiluminescent, or radioactive.

10 The polyclonal or monoclonal antibodies may further be used to isolate HCV particles or antigens by immunoaffinity columns. The antibodies can be affixed to a solid support by, for example, adsorption or by covalent linkage so that the antibodies retain their immunoselective activity. Optionally, spacer groups may be included so that the antigen binding site of the antibody remains accessible. The 15 immobilized antibodies can then be used to bind HCV particles or antigens from a biological sample, such as blood or plasma. The bound HCV particles or antigens are recovered from the column matrix by, for example, a change in pH.

HCV-Specific T cells

20 HCV-specific T cells that are activated by the above-described fusions and E1E2 complexes, including the NS3NS4NS5a fusion protein or NS3NS4NS5aNS5b fusion protein, and the E1E2 complexes, expressed *in vivo* or *in vitro*, preferably recognize an epitope of an HCV polypeptide such as an E1, E2, NS3, NS4, NS5a, NS5b polypeptide, including an epitope of an NS3NS4NS5a fusion protein or an 25 NS3NS4NS5aNS5b fusion protein, or an E1E2 complex. HCV-specific T cells can be CD8⁺ or CD4⁺.

HCV-specific CD8⁺ T cells preferably are cytotoxic T lymphocytes (CTL) which can kill HCV-infected cells that display E1, E2, NS3, NS4, NS5a, NS5b epitopes complexed with an MHC class I molecule. HCV-specific CD8⁺ T cells may 30 also express interferon- γ (IFN- γ). HCV-specific CD8⁺ T cells can be detected by, for example, ⁵¹Cr release assays (see the examples). ⁵¹Cr release assays measure the

ability of HCV-specific CD8⁺ T cells to lyse target cells displaying an E1, E2, E1E2, NS3, NS4, NS5a, NS5b, NS3NS4NS5a, or NS3NS4NS5aNS5b epitope. HCV-specific CD8⁺ T cells which express IFN- γ can also be detected by immunological methods, preferably by intracellular staining for IFN- γ after *in vitro* stimulation with 5 an E1, E2, NS3, an NS4, an NS5a, or an NS5b polypeptide (see the examples).

HCV-specific CD4⁺ cells activated by the above-described E1E2 complexes and fusions, such as an E1 polypeptide, an E2 polypeptide, an E1E2 complex, NS3NS4NS5a or NS3NS4NS5aNS5b fusion protein, expressed *in vivo* or *in vitro*, preferably recognize an epitope of an E1, E2, NS3, NS4, NS5a, or NS5b polypeptide, 10 including an epitope of an E1E2 complex, NS3NS4NS5a or NS3NS4NS5aNS5b fusion protein, that is bound to an MHC class II molecule on an HCV-infected cell and proliferate in response to stimulating E1E2 complexes with NS3NS4NS5a or NS3NS4NS5aNS5b peptides, with or without a core polypeptide.

HCV-specific CD4⁺ T cells can be detected by a lymphoproliferation assay 15 (see examples). Lymphoproliferation assays measure the ability of HCV-specific CD4⁺ T cells to proliferate in response to an E1, E2, NS3, an NS4, an NS5a, or an NS5b epitope.

Methods of Activating HCV-Specific T Cells.

20 The HCV proteins or polynucleotides can be used to stimulate an immune response, such as to activate HCV-specific T cells either *in vitro* or *in vivo*. Activation of HCV-specific T cells can be used, *inter alia*, to provide model systems to optimize CTL responses to HCV and to provide prophylactic or therapeutic treatment against HCV infection. For *in vitro* activation, proteins are preferably 25 supplied to T cells via a plasmid or a viral vector, such as an adenovirus vector, as described above.

Polyclonal populations of T cells can be derived from the blood, and 30 preferably from peripheral lymphoid organs, such as lymph nodes, spleen, or thymus, of mammals that have been infected with an HCV. Preferred mammals include mice, chimpanzees, baboons, and humans. The HCV serves to expand the number of

activated HCV-specific T cells in the mammal. The HCV-specific T cells derived from the mammal can then be restimulated *in vitro* by adding, e.g., HCV E1E2 and NS3NS4NS5a or NS3NS4NS5aNS5b epitopic peptides, with or without a core polypeptide, to the T cells. The HCV-specific T cells can then be tested for, *inter alia*, proliferation, the production of IFN- γ , and the ability to lyse target cells displaying E1E2, NS3NS4NS5a or NS3NS4NS5aNS5b epitopes *in vitro*.

In a lymphoproliferation assay (see examples), HCV-activated CD4 $^{+}$ T cells proliferate when cultured with an NS3, NS4, NS5a, NS5b, NS3NS4NS5a, or NS3NS4NS5aNS5b epitopic peptide, but not in the absence of an epitopic peptide.

10 Thus, particular E1, E2, NS3, NS4, NS5a, NS5b, NS3NS4NS5a and NS3NS4NS5aNS5b epitopes that are recognized by HCV-specific CD4 $^{+}$ T cells can be identified using a lymphoproliferation assay.

Similarly, detection of IFN- γ in HCV-specific CD8 $^{+}$ T cells after *in vitro* stimulation with the above-described HCV proteins, can be used to identify E1, E2, E1E2, NS3, NS4, NS5a, NS5b, NS3NS4NS5a, and NS3NS4NS5aNS5b epitopes that are particularly effective at stimulating CD8 $^{+}$ T cells to produce IFN- γ (see examples).

Further, ^{51}Cr release assays are useful for determining the level of CTL response to HCV. See Cooper *et al.* *Immunity* 10:439-449. For example, HCV-specific CD8 $^{+}$ T cells can be derived from the liver of an HCV infected mammal.

20 These T cells can be tested in ^{51}Cr release assays against target cells displaying, e.g., E1E2, NS3NS4NS5a and/or NS3NS4NS5aNS5b epitopes. Several target cell populations expressing different E1E2, NS3NS4NS5a and/or NS3NS4NS5aNS5b epitopes can be constructed so that each target cell population displays different epitopes of E1E2, NS3NS4NS5a and/or NS3NS4NS5aNS5b. The HCV-specific

25 CD8 $^{+}$ cells can be assayed against each of these target cell populations. The results of the ^{51}Cr release assays can be used to determine which epitopes of E1E2, NS3NS4NS5a and/or NS3NS4NS5aNS5b are responsible for the strongest CTL response to HCV. E1E2 complexes, NS3NS4NS5a fusion proteins or NS3NS4NS5aNS5b fusion proteins, with or without core polypeptides, which

contain the epitopes responsible for the strongest CTL response can then be constructed using the information derived from the ^{51}Cr release assays.

HCV proteins as described above, or polynucleotides encoding such proteins, can be administered to a mammal, such as a mouse, baboon, chimpanzee, or human, 5 to stimulate an immune response, such as to activate HCV-specific T cells *in vivo*. Administration can be by any means known in the art, including parenteral, intranasal, intramuscular or subcutaneous injection, including injection using a biological ballistic gun ("gene gun"), as discussed above.

Preferably, injection of an HCV polynucleotide is used to activate T cells. In 10 addition to the practical advantages of simplicity of construction and modification, injection of the polynucleotides results in the synthesis of a fusion protein in the host. Thus, these immunogens are presented to the host immune system with native post-translational modifications, structure, and conformation. The polynucleotides are 15 preferably injected intramuscularly to a large mammal, such as a human, at a dose of 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 5 or 10 mg/kg.

A composition of the invention comprising the HCV proteins or 20 polynucleotides is administered in a manner compatible with the particular composition used and in an amount which is effective to stimulate an immune response, such as to activate HCV-specific T cells as measured by, *inter alia*, a ^{51}Cr release assay, a lymphoproliferation assay, or by intracellular staining for IFN- γ . The proteins and/or polynucleotides can be administered either to a mammal which is not infected with an HCV or can be administered to an HCV-infected mammal. The 25 particular dosages of the polynucleotides or proteins in a composition will depend on many factors including, but not limited to the species, age, and general condition of the mammal to which the composition is administered, and the mode of administration of the composition. An effective amount of the composition of the invention can be readily determined using only routine experimentation. *In vitro* and 30 *in vivo* models described above can be employed to identify appropriate doses. The amount of polynucleotide used in the example described below provides general guidance which can be used to optimize the activation of HCV-specific T cells either

5 *in vivo* or *in vitro*. Generally, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 5 or 10 mg of an HCV fusion and E1 and E2 polypeptides, such as an E1E2 complex, an NS3NS4NS5a or NS3NS4NS5aNS5b fusion protein or polynucleotide, with or without a core polypeptide, will be administered to a large mammal, such as a baboon, chimpanzee, or human. If desired, co-stimulatory molecules or adjuvants can also be provided before, after, or together with the compositions.

10 Immune responses of the mammal generated by the delivery of a composition of the invention, including activation of HCV-specific T cells, can be enhanced by varying the dosage, route of administration, or boosting regimens. Compositions of the invention may be given in a single dose schedule, or preferably in a multiple dose schedule in which a primary course of vaccination includes 1-10 separate doses, followed by other doses given at subsequent time intervals required to maintain and/or reinforce an immune response, for example, at 1-4 months for a second dose, and if needed, a subsequent dose or doses after several months.

15

Deposits of Strains Useful in Practicing the Invention

20 A deposit of biologically pure cultures of the following strains was made with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA. The accession number indicated was assigned after successful viability testing, and the requisite fees were paid, made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of viable cultures for a period of thirty (30) years from the date of deposit. The organisms will be made available by the ATCC under the terms of the 25 Budapest Treaty, which assures permanent and unrestricted availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 U.S.C. §122 and the Commissioner's rules pursuant thereto (including 37 C.F.R. §1.12 with particular reference to 886 OG 638). Upon the granting of a patent, all restrictions on the availability to the public of 30 the deposited cultures will be irrevocably removed.

These deposits are provided merely as convenience to those of skill in the art, and are not an admission that a deposit is required under 35 U.S.C. §112. The nucleic acid sequences of these genes, as well as the amino acid sequences of the molecules encoded thereby, are incorporated herein by reference and are controlling 5 in the event of any conflict with the description herein. A license may be required to make, use, or sell the deposited materials, and no such license is hereby granted.

	Plasmid	Deposit Date	ATCC No.
10	E1E2-809	August 16, 2001	PTA-3643

III. Experimental

15 Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way. Those of skill in the art will readily appreciate that the invention may be practiced in a variety of ways given the teaching of this disclosure.

20 Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

EXAMPLE 1

25 Production of NS3NS4NS5a Polynucleotides.

A polynucleotide encoding NS3NS4NS5a (approximately amino acids 1027 to 2399, numbered relative to HCV-1) (also termed "NS345a" herein) or NS5a (approximately amino acids 1973 to 2399, numbered relative to HCV-1) was isolated from an HCV. Polynucleotides encoding a methionine residue were ligated to the 5' 30 end of these polynucleotides and the polynucleotides were cloned into plasmid, vaccinia virus, and adenovirus vectors.

Immunization Protocols. In one immunization protocol, mice were immunized with 50 µg of plasmid DNA encoding either NS5a or encoding an NS3NS4NS5a fusion protein by intramuscular injection into the tibialis anterior. A booster injection of 10⁷ pfu of vaccinia virus (VV)-NS5a (intraperitoneal) or 50 µg of 5 plasmid control (intramuscular) was provided 6 weeks later.

In another immunization protocol, mice were injected intramuscularly in the tibialis anterior with 10¹⁰ adenovirus particles encoding an NS3NS4NS5a fusion protein. An intraperitoneal booster injection of 10⁷ pfu of VV-NS5a or an 10 intramuscular booster injection of 10¹⁰ adenovirus particles encoding NS3NS4NS5a was provided 6 weeks later.

EXAMPLE 2

Immunization with DNA encoding an NS3NS4NS5a fusion protein activates HCV-specific CD8⁺ T cells.

15 *⁵¹Cr Release Assay.* A ⁵¹Cr release assay was used to measure the ability of HCV-specific T cells to lyse target cells displaying an NS5a epitope. Spleen cells were pooled from the immunized animals. These cells were restimulated *in vitro* for 6 days with the CTL epitopic peptide p214K9 (2152-HEYYPVGSQQL-2160; SEQ ID NO:1) from HCV-NS5a in the presence of IL-2. The spleen cells were then assayed 20 for cytotoxic activity in a standard ⁵¹Cr release assay against peptide-sensitized target cells (L929) expressing class I, but not class II MHC molecules, as described in Weiss (1980) J. Biol. Chem. 255:9912-9917. Ratios of effector (T cells) to target (B cells) of 60:1, 20:1, and 7:1 were tested. Percent specific lysis was calculated for each effector to target ratio.

25 The results of the assays are shown in Tables 1 and 2. Table 1 demonstrates that immunization with plasmid DNA encoding an NS3NS4NS5a fusion protein activates CD8⁺ T cells which recognize and lyse target cells displaying an NS5a epitope. Surprisingly the NS5a polypeptide of the NS3NS4NS5a fusion protein was able to activate T cells even though the NS5a polypeptide was present in a fusion 30 protein.

Similarly, Table 2 demonstrates that delivery of the NS3NS4NS5a fusion protein to mice by means of an adenovirus vector also activates CD8⁺ T cells which recognize and lyse target cells displaying an HCV NS5a epitope. Thus, immunization with either "naked" (plasmid) DNA encoding an NS3NS4NS5a fusion protein or adenovirus vector-encoded fusion protein can be used to activate HCV-specific T cells.

EXAMPLE 3

10 Immunization with DNA encoding an NS3NS4NS5a fusion protein activates HCV-specific CD8⁺ T cells which express IFN- γ .

Intracellular Staining for Interferon-gamma (IFN- γ). Intracellular staining for IFN- γ was used to identify the CD8⁺ T cells that secrete IFN- γ after *in vitro* stimulation with the NS5a epitope p214K9. Spleen cells of individual immunized mice were restimulated *in vitro* either with p214K9 or with a non-specific peptide for 15 6-12 hours in the presence of IL-2 and monensin. The cells were then stained for surface CD8 and for intracellular IFN- γ and analyzed by flow cytometry. The percent of CD8⁺ T cells which were also positive for IFN- γ was then calculated. The results of these assays are shown in Tables 1 and 2. Table 1 demonstrates that CD8⁺ T cells activated in response to immunization with plasmid DNA encoding an 20 NS3NS4NS5a fusion protein also express IFN- γ . Immunization with an NS3NS4NS5a fusion protein encoded in an adenovirus also results in CD8⁺ HCV-specific T cells which express IFN- γ , although to a lesser extent than immunization with a plasmid-encoded NS3NS4NS5a fusion protein (Table 2).

Table 1. HCV-NS5a-Specific CD8+ T Cells in Mice Immunized with NS5a or NS345a DNA

Table 1. HCV-NS5a-Specific CD8+ T Cells in Mice Immunized with NS5a or NS345a DNA						
⁵¹ Cr Release Assay			Intracellular Staining for IFN- γ			
E:T ratio	Percent Specific Lysis of Targets*		Percent of CD8+ T Cells Positive for IFN-g ^{**}			
	NS5a DNA	NS345a DNA	NS5a DNA	NS345a DNA	NS5a DNA	NS345a DNA
p214K9	-	p214K9	-	p214K9	-	p214K9
60:1	77	5	66	6		-
20:1	61	4	49	2	1.74	0.26
7:1	29	1	29	4		

*Target cells (L929) were pulsed with p214K9 or media alone and labeled with ^{51}Cr .

***Spleen cells were cultured with p214K9 or media alone for 12 hours in the presence of monensin.

p214K9 is a CTL epitopic peptide (2152-HEYPVGSQ-2160, SEQ ID NO:1) from HCV-NS5a

Table 2. HCV-NS5a-Specific CD8+ T Cells Primed by Adenovirus or DNA Encoding for NS345a

E:T ratio	51Cr Release Assay			Intracellular Staining for IFN- γ		
	NS345a Adeno	NS345a DNA	Percent Specific Lysis of Targets*	NS345a Adeno	Percent of CD8+ T Cells Positive for IFN- γ **	NS345a DNA
60:1	p214K9	-	p214K9	-	p214J	p214J
	76	2	55	5		
20:1	85	2	22	3	3.24	0.13
7:1	62	<1	10	3		0.25
						0.09

*Target cells (L929) were pulsed with p214K9 or p214J and labeled with ⁵¹Cr.

**Spleen cells were cultured with p214K9 or p214J for 12 hours in the presence of monensin.

p214K9 is a CTL epitope peptide (2152-HEYPVGSQI-2160, SEQ ID NO:1) from HCV-NS5a
 p214J is a control peptide (10 mer) from HCV-NS5a

EXAMPLE 4

Immunization with DNA encoding an NS3NS4NS5a fusion protein stimulates proliferation of HCV-specific CD4⁺ T cells.

Lymphoproliferation assay. Spleen cells from pooled immunized mice were 5 depleted of CD8⁺ T cells using magnetic beads and were cultured in triplicate with either p222D, an NS5a-epitopic peptide from HCV-NS5a (2224-AELIEANLLWRQEMG-2238; SEQ ID NO:2), or in medium alone. After 72 hours, cells were pulsed with 1 μ Ci per well of ³H-thymidine and harvested 6-8 hours later. Incorporation of radioactivity was measured after harvesting. The mean cpm was 10 calculated.

As shown in Table 3, immunization with a plasmid-encoded NS3NS4NS5a fusion protein stimulates proliferation of CD4⁺ HCV-specific T cells. Immunization with an adenovirus vector encoding the fusion protein also resulted in stimulated proliferation of CD4⁺ HCV-specific T cells (Table 4).

15

20

Table 3. HCV-NS5a-Specific CD4+ T Cells in Mice Immunized with NS5a or NS345a DNA			
Mean CPM			
NS5a DNA		NS345a DNA	
p222D	media	p222D	media
4523	740	4562	861
(x6.1)		(x5.3)	

p222D is a CD4⁺ epitopic peptide (aa: 2224-AELIEANLLWRQEMG-2238, SEQ ID NO:2) from HCV-NS5a 25

Table 4. HCV-NS5-Specific CD4+ T Cells Primed by Adenovirus or DNA Encoding for NS345a

Mean CPM			
NS345a Adeno		NS345a DNA	
	p222D	media	p222D
5	896	357	1510
	(x2.5)		(x3.9)

10 p222D is a CD4+ epitopic peptide (aa: 2224-AELIEANLLWRQEMG-2238, SEQ ID NO:2) from HCV-NS5a

EXAMPLE 5

Efficiency of NS345a-encoding DNA Vaccine Formulations to prime CTLs in mice.

15 Mice were immunized with either 10-100 µg of plasmid DNA encoding NS345a fusion protein as described in Example 1, with PLG-linked DNA encoding NS345a, described below, or with DNA encoding NS345a, delivered via electroporation (see, e.g., U.S. Patent Nos. 6,132,419; 6,451,002, 6,418,341, 6233,483, U.S. Patent Publication No. 2002/0146831; and International Publication No. WO/0045823, for this delivery technique). The immunizations were followed by a booster injection 6 weeks later of 1×10^7 pfu vaccinia virus encoding NS5a, plasmid DNA encoding NS345a or plasmid DNA encoding NS5a each as described in Example 1.

20 *PLG-delivered DNA.* The polylactide-co-glycolide (PLG) polymers were obtained from Boehringer Ingelheim, U.S.A. The PLG polymer used in this study was RG505, which has a copolymer ratio of 50/50 and a molecular weight of 65 kDa (manufacturers data). Cationic microparticles with adsorbed DNA were prepared using a modified solvent evaporation process, essentially as described in Singh et al., *Proc. Natl. Acad. Sci. USA* (2000) 97:811-816. Briefly, the microparticles were 25 prepared by emulsifying 10 ml of a 5% w/v polymer solution in methylene chloride with 1 ml of PBS at high speed using an IKA homogenizer. The primary emulsion 30 was then added to 50ml of distilled water containing cetyl trimethyl ammonium

bromide (CTAB) (0.5% w/v). This resulted in the formation of a w/o/w emulsion which was stirred at 6000 rpm for 12 hours at room temperature, allowing the methylene chloride to evaporate. The resulting microparticles were washed twice in distilled water by centrifugation at 10,000 g and freeze dried. Following preparation, 5 washing and collection, DNA was adsorbed onto the microparticles by incubating 100 mg of cationic microparticles in a 1mg/ml solution of DNA at 4 C for 6 hours. The microparticles were then separated by centrifugation, the pellet washed with TE buffer and the microparticles were freeze dried.

10 CTL activity and IFN- γ expression were measured by ^{51}Cr release assay or intracellular staining as described in examples 2 and 3 respectively. The results are shown in Table 5.

Results demonstrate that immunization using plasmid DNA encoding for NS345a to prime mice results in activation of CD8+ HCV specific T cells.

Table 5: Efficiency of NS345a-Encoding DNA Vaccine Formulations to Prime CTLs in Mice

				ICS for IFN-gamma (%CD8+ cells that are IFN-g ⁺)						
5	NS345a DNA Vaccines	Boost	Mean	SddevP	# of mice tested	% respon- d- ing	# of expts	fold increase vs. 'naked' DNA	CTL activ- i- ty?	
10	NS345a DNA	VVNS5a	1.02	1.70	41	68%	10	N/A	YES	
	NS345a DNA	NS345a DNA	0.02	0.04	22	5%	5	N/A	YES	
15	NS345a DNA	NS5a DNA	0.22	0.21	24	63%	5	N/A	YES	
	NS345a DNA eV (electro- poration)	VVNS5a	5.00	4.36	7	100%	2	4.90	YES	
20	PLGNS34 5a DNA	VVNS5a	2.65	2.54	6	100%	2	2.60	YES	
	PLGNS34 5a DNA	NS5a DNA	0.33	0.24	15	80%	3	1.50	YES	

EXAMPLE 6

25 *Immunization routes and replicon particles SINCR (DC+) encoding for NS345a*
 Alphavirus replicon particles, for example, SINCR (DC+) were prepared as
 described in Polo et al., *Proc. Natl. Acad. Sci. USA* (1999) **96**:4598-4603. Mice were
 injected with 5×10^6 IU SINCR (DC+) replicon particles encoding for NS345a
 intramuscularly (IM) as described in Example 1, or subcutaneously (S/C) at the base of the
 30 tail (BoT) and foot pad (FP), or with a combination of 2/3 of the DNA delivered via IM
 administration and 1/3 via a BoT route. The immunizations were followed by a booster
 injection of vaccinia virus encoding NS5a as described in Example 1.

IFN- γ expression was measured by intracellular staining as described in Example 3.
 The results are shown in Table 6. The results demonstrate that immunization via SINCR
 35 (DC+) replicon particles encoding for NS345a by a variety of routes results in CD8+ HCV
 specific T cells which express IFN- γ .

Table 6: Immunization Routes and SINCR (DC+) Replicon Particles Encoding NS345a
(all mice VvNNS5a challenged)

Vaccines	Immunization Route	ICS for IFN-gamma (%CD8+ cells that are IFN-g+)				% responding mice
		Mean	Sd/DevP	# of mice tested	# of expts	
SINCR (DC+) 5X10 ⁶	100% IM (ta)	1.11	0.63	3	1	100%
SINCR (DC+) 5X10 ⁶	100% S/C (BoT + FP)	0.62	0.29	3	1	100%
SINCR (DC+) 5X10 ⁶	2/3 IM (ta) + 1/3 S/C (BoT)	2.43	2.00	3	1	100%

EXAMPLE 7*SINCR (DC+) vs SINCR (LP) replicon particles encoding for NS345a*

Alphavirus replicon particles, for example, SINCR (DC+) and SINCR (LP) were prepared as described in Polo et al., *Proc. Natl. Acad. Sci. USA* (1999) 96:4598-4603. Mice 5 were immunized with 1×10^3 to 1×10^7 IU of SINCR (DC+) or SINCR (LP) replicon particles encoding for NS345a, by intramuscular injection into the tibialis anterior, followed by a booster injection of 10^7 pfu vaccinia virus encoding NS5a at 6 weeks.

IFN- γ expression was measured by intracellular staining as described in Example 3. Administration of an increase in the number of SINCR (DC+) replicon particles encoding 10 NS345a resulted in an increase in % of CD8+ T cells expressing IFN- γ .

EXAMPLE 8*Alphavirus replicon priming, followed by various boosting regimes.*

Alphavirus replicon particles, for example, SINCR (DC+) were prepared as 15 described in Polo et al., *Proc. Natl. Acad. Sci. USA* (1999) 96:4598-4603. Mice were primed with SINCR (DC+), 1.5×10^6 IU replicon particles encoding NS345a, by intramuscular injection into the tibialis anterior, followed by a booster of either 10-100 μ g of plasmid DNA encoding for NS5a, 10^{10} adenovirus particles encoding NS345a, 1.5×10^6 IU SINCR (DC+) replicon particles encoding NS345a, or 10^7 pfu vaccinia virus encoding 20 NS5a at 6 weeks.

IFN- γ expression was measured by intracellular staining as described in Example 3. The results are shown in Table 7. The results demonstrate that boosting with vaccinia virus 25 encoding NS5a DNA results in the strongest generation of CD8+ HCV specific T cells which express IFN- γ . Boosting with plasmid encoding NS5a DNA also results in a good response, while lesser responses are noted with adenovirus NS345a or SINCR DC+ boosted animals.

Table 7: Alphavirus Replicon Particle Priming, Followed by Various Boosting Regimens

		ICS for IFN-gamma (%CD8+ cells that are IFN-g+)					
5	Vaccines	Boost	Mean	SdtdevP	# of mice tested	# of expts	% responding mice
	SINCR (DC+) 1.5X10 ⁶	NS5a DNA	0.46	0.36	4	1	75%
10	SINCR (DC+) 1.5X10 ⁶	Adeno NS345a (10X10 ¹⁰)	0.04	0.04	4	1	25%
	SINCR (DC+) 1.5X10 ⁶	SINCR (DC+) 1.5X10 ⁶	0.06	0.06	8	2	25%
	SINCR (DC+) 1.5X10 ⁶	VVNS5a (1X10 ⁷)	2.43	2.45	4	1	100%

15

EXAMPLE 9*Alphaviruses expressing NS345a*

Alphavirus replicon particles, for example, SINCR (DC+) and SINCR (LP) were prepared as described in Polo et al., *Proc. Natl. Acad. Sci. USA* (1999) 96:4598-4603. Mice were immunized with 1 x 10² to 1 x 10⁶ IU SINCR (DC+) replicons encoding NS345a via a combination of delivery routes (2/3 IM and 1/3 S/C) as well as by S/C alone, or with 1 x 10² to 1 x 10⁶ IU SINCR (LP) replicon particles encoding NS345a via a combination of delivery routes (2/3 IM and 1/3 S/C) as well as by S/C alone.

The immunizations were followed by a booster injection of 10⁷ pfu vaccinia virus encoding NS5a at 6 weeks.

IFN- γ expression was measured by intracellular staining as described in Example 3. The results are shown in Figure 6. The results indicate activation of CD8+ HCV specific T cells.

30

EXAMPLE 10*Efficiency of NS5a encoding DNA vaccine formulations to prime CTLs in mice*

5 Mice were immunized with either 10-100 µg of plasmid DNA encoding NS5a as described in Example 1 or with PLG-linked DNA encoding NS5a as described in Example 5. The immunizations were followed by a booster injection at 6 weeks of either 10-100 µg of plasmid DNA encoding for NS5a, 10¹⁰ adenovirus particles encoding NS345a, 1.5 x 10⁶ IU SINCR (DC+) replicon particles encoding NS345a, or 10⁷ pfu vaccinia virus encoding NS5a.

10 CTL activity and IFN- γ expression were measured by the methods described in Examples 2 and 3.

The results are shown in Table 8. The results demonstrate that priming with plasmid DNA encoding for NS5a or PLG-linked DNA encoding NS5a results in activation of CD8+ HCV specific T cells.

15

Table 8: Efficiency of NS5a-Encoding DNA Vaccine Formulations to Prime CTLs in Mice

NS5a Vaccines	Boost	Mean	Sd/DevP	ICS for IFN-gamma (%CD8+ cells that are IFN- g+)		# of expts	fold increase vs. 'naked' DNA	CTL activity ?
				# of mice tested	% respond- ing			
VVNS5a		1.67	1.49	8	100%	3	N/A	YES
NS5a DNA	NS5a DNA	0.17	0.09	12	83%	3	N/A	YES
PLGNSS5a DNA	NS5a DNA	0.22	0.09	9	100%	2	1.29	YES
NS5a DNA	AdenoNS 345a	0.10	0.08	4	50%	1	N/A	NO
NS5a DNA	SINCRNS 345a	0.20	0.17	4	75%	1	N/A	YES

EXAMPLE 11*Efficiency of NS345b-encoding DNA vaccine formulations to prime CTLs in mice*

5 Mice were immunized with 10-100 µg of plasmid DNA encoding NS345b by intramuscular injection to the tibialis anterior or with PLG linked DNA encoding NS5a as described in Example 5. The immunizations were followed by a booster injection of plasmid DNA encoding for NS5a as described in Example 1.

10 CTL activity and IFN- γ expression were measured by the methods described in Examples 2 and 3.

The results are shown in Table 9. The results demonstrate that priming with plasmid DNA encoding NS345b or PLG-linked NS345b results in activation of CD8+ HCV specific T cells.

Table 9: Efficiency of NS345b-Encoding DNA Vaccine Formulations to Prime CTLs in Mice

NS345 DNA Vaccines	Boost	Mean	Sd/DevP	ICS for IFN-gamma (%CD8+ cells that are IFN-g+)		# of expts	fold increase vs. 'naked' DNA	CTL activity?
				# of mice tested	% responding			
NS345 DNA	NS5a DNA	0.18	0.16	15	60%	3	N/A	YES
PLGNNS345 DNA	NS5a DNA	0.30	0.33	14	57%	3	1.67	YES

EXAMPLE 12*Administration of DNA via separate plasmids*

5 Mice were immunized with 100 µg plasmid DNA encoding for NS345a or with 100 µg PLG-linked DNA encoding NS345a. Additionally, separate DNA plasmids encoding NS5a, NS34a, and NS4ab (33.3 µg each) were administered concurrently to another group of mice. Finally, PLG-linked DNA encoding NS5a, NS34a, and NS4ab (33.3 µg each) were administered concurrently to another group of mice. The immunizations were followed by a booster injection of 1x10⁷ pfu vaccinia virus encoding NS5a, 6 weeks post first immunization.

10 IFN- γ expression was measured by the method described in Example 3. The results are shown in Figure 7. The results demonstrate a particularly vigorous response in the activation of CD8+ HCV specific T cells when the DNA is broken down into smaller sub units and linked to PLG.

EXAMPLE 13*Immunogenicity of NS345Core₁₂₁-ISCOMS in Mice*

Groups of 10 C57 black mice were immunized IM at 0, 21 and 60 days with the formulations shown in Table 10. The NS345Core₁₂₁-PLGdss group received a vaccine dose of 50 µl in each leg whereas the other vaccine groups received a vaccine dose of 50 µl in one leg.

20 NS345Core₁₂₁-ISCOMS were comprised of amino acids 1242 to 3011 and 1-121 and the HCV polyprotein, numbered relative to HCV-1 and were adsorbed to ISCOMS with a ratio of protein to QH of approximately 8:1, using standard techniques. See, e.g., International Publication No. WO 01/37869A.

25 Core-ISCOMS including an HCV core protein fragment from the region spanning amino acid positions 1-191 of the HCV polyprotein, numbered relative to HCV-1, with a ratio of protein to QH of 1:1, were produced using standard techniques. See, e.g., International Publication No. WO 01/37869A.

30 NS345Core₁₂₁ was formulated in 0.1% SDS in PBS and contained DTT. Protein was diluted in PBS and mixed 1:1 with MF59 (see, Ott et al., "MF59 --

Design and Evaluation of a Safe and Potent Adjuvant for Human Vaccines" in *Vaccine Design: The Subunit and Adjuvant Approach* (Powell, M.F. and Newman, M.J. eds.) Plenum Press, New York (1995) pp. 277-296; and U.S. Patent No. 6,299,884) prior to immunization.

5 For NS345Core₁₂₁-PLGdss, PLG microparticles produced as described above were treated with 3-(trimethylsilyl)-1-propanesulfonic acid (DSS) to enhance adsorption of antigen. DSS is commercially available from, e.g., Sigma Chemical Co., St. Louis, MO. NS345Core₁₂₁ was adsorbed thereto using standard techniques (see, International Publication No. WO 00/050006). The NS345Core₁₂₁-PLGdss was
10 mixed with MF59 prior to immunization.

As shown in Table 10, NS345Core₁₂₁-ISCOMS produced antibody response only to NS5 in immunized C57 black mice. Higher levels of antibodies to NS5 were produced in mice immunized with NS345Core₁₂₁ adjuvanted with MF59, however no antibody response to core, NS3 or NS4 was produced with this adjuvant either.

15 Mice immunized with Core-ISCOMS produced antibodies to core. In contrast, NS345Core₁₂₁-PLGdss immunized mice produced significantly higher antibodies to NS5 than NS345Core₁₂₁-ISCOMS. In addition, NS345Core₁₂₁-PLGdss immunized mice produced antibodies to NS3 and some antibody response to core, but no antibodies to NS4.

20

5

Table 10. Immunogenicity of NS345Core₁₂₁-Iscoms in Mice. Geometric mean EIA antibody titers to core and nonstructural proteins are shown. The number of responding mice per group are also listed.

10

15

Vaccine	IM Protein Dose (μg) ^a	Anti-Core Antibody EIA GMT	Anti-C33C (NS3) Antibody EIA GMT	Anti-C100 (NS4) Antibody EIA GMT	Anti-NS5 Antibody EIA GMT
NS345Core ₁₂₁ ISCOMS	6.0, 6.0, 6.0 ^b	<10 (0/10)	<10 (0/10)	<10 (0/10)	31 (7/10)
Core- ISCOMS	6.0, 6.0, 6.0 ^c	188 (9/10)	<10 (1/10)	<10 (0/10)	<10 (2/10)
NS345Core ₁₂₁ MF59	6.0, 6.0, 6.0	<10 (2/9)	<10 (1/9)	<10 (0/9)	279 (9/)
NS345Core ₁₂₁ PLG-dss/MF59	10, 10, 10	5 (6/10)	50 (9/10)	<10 (2/10)	419 (9/9)

^aGroups of 10 C57 black mice were immunized IM at 0, 21 and 60 days. Serum was obtained after the last immunization. The NS345 Core₁₂₁-PLGdss group received vaccine dose of 50 μl in each leg whereas the other vaccine groups received vaccine dose of 50 μl in one leg.

^bThe ratio of protein to QH was approximately 8:1.

^cThe ratio of protein to QH was approximately 1:1.

EXAMPLE 14

25

Immunogenicity of Different Formulations of NS345Core₁₂₁ or NS345 in Mice

30

Groups of 10 C57 black mice were immunized IM at 0, 30 and 60 days with the formulations shown in Tables 11 and 12. For the studies shown in Table 11, the NS345 and NS345Core₁₂₁ protein concentration was 10 μg per dose, and for those in Table 12, the concentration was 5 μg per dose.

For PLG-NS345 (amino acids 1242 to 3011 of the HCV polyprotein) and PLG-NS345Core₁₂₁ (amino acids 1242-3011 and 1-121 of the HCV polyprotein), PLG microparticles were prepared and NS345 or NS345Core₁₂₁ were adsorbed thereto using standard techniques, as described above.

5 For PLG-NS345 + PLG-CTAB-E1E2 DNA, PLG microparticles were prepared and NS345 was adsorbed to the microparticles as described above. E1E2 DNA was produced as follows. Mammalian expression plasmid pMH-E1E2-809 (Figure 4, ATCC Deposit No. PTA-3643) encodes an E1E2 fusion protein which includes amino acids 192-809 of HCV 1a (see, Choo et al., *Proc. Natl. Acad. Sci. USA* (1991) 88:2451-2455). Chinese Hamster Ovary (CHO) cells were used for expression of the HCV E1E2 sequence from pMH-E1E2-809. In particular, CHO DG44 cells were used. These cells, described by Uraub et al., *Proc. Natl. Acad. Sci. USA* (1980) 77:4216-4220, were derived from CHO K-1 cells and were made dihydrofolate reductase (dhfr) deficient by virtue of a double deletion in the dhfr gene. DG44 cells were transfected with pMH-E1E2-809. The transfected cells were grown in selective medium such that only those cells expressing the dhfr gene could grow (Sambrook et al., *supra*). Isolated CHO colonies were picked (~800 colonies) into individual wells of a 96-well plate. From the original 96-well plates, replicates were made to perform expression experiments. The replicate plates were grown until 10 the cells made a confluent monolayer. The cells were fixed to the wells of the plate and permeabilized using cold methanol. Anti-E1 and anti-E2 antibodies, 3D5C3 and 3E5-1 respectively, were used to probe the fixed cells. After adding an anti-mouse HRP conjugate, followed by substrate, the cell lines with the highest expression were determined. The highest expressing cell lines were then expanded to 24-well cluster 15 plates. The assay for expression was repeated, and again, the highest expressing cell lines were expanded to wells of greater volume. This was repeated until the highest expressing cell lines were expanded from 6-well plates into tissue culture flasks. At 20 this point there was sufficient quantity of cells to allow accurate count and harvest of the cells, and quantitative expression assays were done. An ELISA was performed 25 on the cell extract, to determine high expressors.

To produce the PLG-CTAB-E1E2 DNA, PLG microparticles were treated with CTAB as described above (see, International Publication No. WO 00/050006).

For PLG-NS345Core₁₂₁ + E1E2 DNA PLG-NS345Core₁₂₁ and E1E2 DNA were produced as described above.

5 For PLG-NS345 or PLG-NS345Core₁₂₁ + MF59, PLG-NS345 or PLG-NS345Core₁₂₁ was combined with MF59 as described above.

For PLG-NS345 or PLG-NS345Core₁₂₁ + CTAB-CpG, NS345 or NS345Core₁₂₁ was adsorbed to PLG as described above. The CpG molecule used was 5'-TCCATGACGTTCCCTGACGTT-3' and this was treated with CTAB, as 10 described above.

For PLG-NS345 or PLG-NS345Core₁₂₁ + QS21, the saponin adjuvant QS21 was combined with the PLG-HCV proteins.

For PLG-NS345 or PLG-NS345Core₁₂₁ + CTAB-CpG + MF59, the various components, as described above, were combined.

15 The remaining adjuvants used in the studies and shown in the tables are self-explanatory.

The results of these studies are shown in Tables 11 and 12. As can be seen in Table 11, none of the formulations produced antibody responses to core, NS3 or NS4 antigens. However, PLG-NS345+CTAB-CPG in MF59 produced the highest 20 antibody titers to NS5. PLG-NS345Core₁₂₁+QS21, PLG-NS345+CTAB-CPG, PLG-NS345Core₁₂₁+CTAB-CPG, and PLG-NS345 + QS21 produced moderate antibody titers to NS5. The other formulations produced very low antibody titers to NS5.

As can be seen in Table 12, NS345Core₁₂₁/MF59/MPL and NS345Core₁₂₁/MF59/CpG formulations produced very high antibody titers to 25 NS345Core₁₂₁. NS345Core₁₂₁/MF59, NS345/MF59/CpG, and NS345Core₁₂₁/MF59/Chol/QS21 formulations produced moderate antibody titers to NS345Core₁₂₁. The other formulations produced very low or no antibody titers to NS345Core₁₂₁.

Table 11. Immunogenicity of different formulations of HCV NS345Core₁₂₁ or NS345 in Mice. Geometric mean EIA antibody titers to core and nonstructural proteins are shown.

	Vaccine ^a	Anti-Core Antibody EIA GMT	Anti-C33C (NS3) Antibody EIA GMT	Anti-C100 (NS4) Antibody EIA GMT	Anti-NS5 Antibody EIA GMT
5	PLG-NS345	<10	<10	<10	10
	PLG-NS345Core ₁₂₁	<10	<10	<10	15
10	PLG-NS345+PLG-CTAB-E1E2 DNA	<10	11	<10	23
	PLG-NS345Core ₁₂₁ +E1E2 DNA	<10	<10	<10	20
15	PLG-NS345+MF59	<10	<10	<10	70
	PLG-NS345Core ₁₂₁ +MF59	<10	<10	<10	26
20	PLG-NS345+CTAB-CPG	<10	<10	<10	350
	PLG-NS345Core ₁₂₁ +CTAB-CPG	<10	<10	<10	271
25	PLG-NS345+QS21	<10	<10	<10	201
	PLG-NS345Core ₁₂₁ +QS21	<10	<10	<10	505

	PLG-NS345 + CTAB-CPG + MF59	<10	<10	<10	1471
5	PLG- NS345Core ₁₂₁ + CTAB+MF59	<10	<10	<10	63

*Groups of 10 C57 black mice were immunized IM at 0, 30 and 60 days. Serum was obtained after the last immunization. The NS345 or NS345Core₁₂₁ protein concentration was 10 µg per dose.

Table 12. Immunogenicity of different formulations of HCV NS345Core₁₂₁ or HCV NS345 in Mice. Geometric mean EIA antibody titers to NS345Core₁₂₁ protein are shown.

	Vaccine ^a	Anti-NS345Core ₁₂₁ Antibody EIA GMT
5	NS345Core ₁₂₁ /MF59	328
	NS345Core ₁₂₁ /MF59/CpG	7,926
	NS34a+NS5B+Core/MF59	12
	NS34a+NS5B+Core/MF59/CpG	5
10	PLG-NS345Core ₁₂₁ /MF59	<10
	PLG-NS345Core ₁₂₁ /MF59/CpG	<10
	PLG-NS345Core ₁₂₁ /PLG-CpG	9
	NS345Core ₁₂₁ /alum phosphate	34
15	NS345Core ₁₂₁ /alum phosphate//CpG	950
	NS345/MF59/CpG	511
	PLG-NS345/PLG/CpG	117
	NS345Core ₁₂₁ /MF59/MPL	10,292
20	NS345Core ₁₂₁ /MF59/Chol/QS21	698
	NS345Core ₁₂₁ /Alum phosphate/MPL	23

*Groups of 10 C57 black mice were immunized IM at 0, 30 and 60 days. Serum was obtained after the last immunization. The NS345 or NS345Core₁₂₁ protein concentration was 5 µg per dose.

EXAMPLE 15

Lymphoproliferative Response of Different Formulations of NS345Core₁₂₁ or NS34A + NS35B + Core in Mice

Groups of 8 C57 black mice were immunized IM at 0, 30 and 60 days with 5 the formulations shown in Table 13 and are as described above. Spleens were obtained after the last immunization. The NS345Core₁₂₁ protein concentration was 25 µg per dose. The NS34a, NS5b and core doses were 3 µg each.

The results of this study are shown in Table 13. As can be seen, 10 NS345Core₁₂₁/Alum/CpG, PLG-NS345Core₁₂₁/PLG/CpG, NS34a+NS5B+Core/MF59/CpG and PLG-NS345Core₁₂₁/MF59/CpG formulations demonstrated strong LPA responses to NS5, NS34 and core antigens. The NS345Core₁₂₁/MF59 formulation also produced a strong LPA response to NS5 and NS34. Core was not tested.

Moderate LPA responses were observed to NS5, NS34 and Core antigens with PLG- 15 NS345Core₁₂₁/MF59 and NS34a +NS5B + Core/MF59 formulations. The NS345Core₁₂₁/MF59/CpG formulation may not have been administered properly in that no LPA response was observed in this experiment. In a subsequent experiment as shown in Table 14, an LPA was observed to this formulation.

Groups of 8 C57 black mice were immunized once IM with the formulations 20 shown in Table 14, produced as described above. Draining lymph nodes were obtained.

The NS345Core₁₂₁ protein concentration was 25 µg per dose.

The results of this study are shown in Table 14. As can be seen in Table 14, 25 all the formulations tested produced a strong LPA response to NS5, NS34 and Core as well as the NS345Core₁₂₁.

5

Table 13. Lymphoproliferative response of different formulations of HCV NS345Core₁₂₁ or NS34A+NS5B+Core in Mice. LPA responses (cpm) to core and nonstructural proteins are shown. The number of mice in each group responding is also indicated in parentheses.

10

15

20

Vaccine ^a	SOD-NS5	SOD-C200 (NS34)	SOD-C22-3 (Core)	HIV-2 env (background control)
NS345Core ₁₂₁ /MF59	2250 (6/8)	1800 (4/8)	ND	144
NS345Core ₁₂₁ /MF59/CpG	80	80	ND	138
PLG-NS345Core ₁₂₁ /MF59	560 (2/8)	120 (2/8)	510 (2/8)	93
PLG-NS345Core ₁₂₁ /MF59/CpG	1600 (6/8)	1500 (6/8)	620 (8/8)	75
NS34a+NS5B+Core/MF59	564 (8/8)	710 (8/8)	265 (8/8)	76
NS34a+NS5B+Core/MF59/CpG	1523 (8/8)	885 (8/8)	446 (6/8)	67
PLG-NS345Core ₁₂₁ /PLG/CpG	3675 (8/8)	2860 (8/8)	370 (8/8)	88
NS345Core ₁₂₁ /Alum/CpG	8450 (8/8)	7940 (8/8)	1040 (6/8)	82

^aGroups of 8 C57 black mice were immunized IM at 0, 30 and 60 days. Spleens were obtained after the last immunization. The NS345Core₁₂₁ protein concentration was 25 µg per dose. The NS34a, NS5B and Core doses were 3 µg each.

25

Table 14. Lymphoproliferative response of different formulations of HCV NS345Core₁₂₁ in Mice. The LPA responses (cpm) from an average of three consecutive experiments to core and nonstructural proteins are shown.

Vaccine ^a	SOD-NS5	SOD-C200 (NS34)	SOD-C22-3 (Core)	NS345Core ₁₂₁	HIV-2 env (background control)
NS345Core ₁₂₁ /MF59	8900	8300	3000	20900	890
NS345Core ₁₂₁ /MF59/CpG	1890	1628	1200	20100	623
PLG-NS345Core ₁₂₁ /MF59	10700	12900	1800	23700	818
PLG-NS345Core ₁₂₁ /MF59/CpG	3600	4690	1660	27300	911
PLG-NS345Core ₁₂₁	4500	4660	760	18150	315
PLG-NS345Core ₁₂₁ /PLG/CpG	7750	5300	1250	22980	450
NS345Core ₁₂₁ /Alum	3050	3725	744	11300	390
NS345Core ₁₂₁ /Alum/CpG	4130	4670	600	20660	480

^aGroups of 8 C57 black mice were immunized once IM. Draining lymph nodes were obtained. The NS345Core₁₂₁ protein concentration was 25 µg per dose.

EXAMPLE 16*Immunogenicity of Recombinant HCV Protein Vaccines Adjuvanted with ISCOMS in Rhesus Macaques*

5 The safety and immunogenicity of HCV proteins completed with the adjuvant, Iscomatrix, was studied in Rhesus macaques. Three groups made up of four animals each were immunized IM as detailed below at week 0, 4 and 8 weeks. Vaccines were prepared as described above. The ISCOMS used lacked QH-A.

10

Group Number	n	Vaccine	Delivery
1	4	Core-ISCOM (50 µg in 1 ml)	0.5 ml R Leg 0.5 ml L Leg
2	4	NS345Core ₁₂₁ -ISCOM (1 mg in 1 ml)	0.5 ml R Leg 0.5 ml L Leg
3	4	Core-ISCOM (25 µg in 0.5 ml) and NS5b-ISCOM (50 µg in 0.35 ml)	0.5 ml Core-ISCOM R Leg 0.35 ml NS5b-ISCOM L Leg

15 Bleeds occurred as follows and immunogenicity was determined by CTL assays, lymphoproliferation assays, FACS analysis and antibody response a previously described (Palakos, et al. (2001) *J. of Immunology* 166:3589).

Week	Bleed date	Immunized
-10		
-1	X	
5	0	X
	2	X
	4	X
	6	X
	8	X
10	X	

The immunogenicity of the different HCV recombinant protein vaccines is shown in Tables 15-17.

5 Table 15. The Immunogenicity of HCV Core-ISCOMS vaccine two weeks post 2nd immunization and post 3rd immunization as assessed by CTL assays, CD8+ FACS analysis, LPA stimulation index and CD4+ FACS analysis

Macaque #	2 weeks post 3°									
	CD8+ ICS (CTL)					CD4+ ICS (LPA SI)				
	C	NS3	NS4	NS5a	NS5b	C	NS3	NS4	NS5a	NS5b
X020	- (-)					+(-)				
N001	- (-)					+(-)				
N086	- (-)					+(-)				
X010	- (-)					+(11)				

Macaque #	2 weeks post 2°									
	CD8+ ICS (CTL)					CD4+ ICS (LPA SI)				
	C	NS3	NS4	NS5a	NS5b	C	NS3	NS4	NS5a	NS5b
X020	- (-)					+(-)				
N001	- (-)					-(8)				
N086	- (-)					+(-)				
X010	- (-)					+(-)				

Table 16. The Immunogenicity of HCV NS345Core₁₂₁-ISCOMS vaccine two weeks post 2nd immunization and post 3rd immunization as assessed by CTL assays, CD8+ FACS analysis, LPA stimulation index and CD4+ FACS analysis

Macaque #	2 weeks post 3°									
	CD8+ ICS (CTL)					CD4+ ICS (LPA SI)				
	C	NS3	NS4	NS5a	NS5b	C	NS3	NS4	NS5a	NS5b
X016	- (-)	+(+)	-(-)	-(-)	-(-)	-(-)	+(-)	-(-)	+/-(-)	+/-(-)
X008	- (-)	-(-)	-(-)	-(-)	-(-)	-(-)	-(-)	-(-)	-(5)	-(-)
X021	- (-)	-(-)	-(-)	-(-)	-(-)	-(-)	-(-)	-(-)	-(-)	-(-)
X023	+/- (-)	-(-)	-(-)	+/-(-)	-(-)	-(-)	+/-(-)	-(-)	-(-)	-(-)

Macaque #	2 weeks post 2°									
	CD8+ ICS (CTL)					CD4+ ICS (LPA SI)				
	C	NS3	NS4	NS5a	NS5b	C	NS3	NS4	NS5a	NS5b
X016	- (-)	+(+)	-(-)	+(-)	+(+)	-(-)	+(-)	+/-(-)	+(-)	+(-)
X008	+ (-)	+(+)	-(-)	+(-)	+(+)	-(-)	+(-)	-(-)	+(-)	+(-)
X021	- (-)	-(-)	+/-(-)	-(-)	+/-(-)	-(-)	-(-)	+/-(-)	-(-)	-(-)
X023	+/- (+)	+(+)	-(-)	+/-(-)	+(-)	-(-)	+(-)	-(-)	-(-)	7

5 Table 17. The Immunogenicity of HCV Core-ISCOMS + NS5b-ISCOMS vaccine two weeks post 2nd immunization and post 3rd immunization as assessed by CTL assays, CD8+ FACS analysis, LPA stimulation index and CD4+ FACS analysis

Macaque #	2 weeks post 3°									
	CD8+ ICS (CTL)					CD4+ ICS (LPA SI)				
	C	NS3	NS4	NS5a	NS5b	C	NS3	NS4	NS5a	NS5b
X022	+(-)				-(-)	-(8)				+/- (11)
X014	-(-)				-(-)	+(6)				+(11)
N154	-(-)				-(-)	-(-)				+(-)
N173	-(-)				-(-)	-(-)				+/- (-)

Macaque #	2 weeks post 2°									
	CD8+ ICS (CTL)					CD4+ ICS (LPA SI)				
	C	NS3	NS4	NS5a	NS5b	C	NS3	NS4	NS5a	NS5b
X022	-(-)				-(+)	-(-)				-(-)
X014	+(-)				+/- (-)	-(-)				+(-)
N154	-(-)				-(-)	-(6)				+(8)
N173	-(-)				-(-)	+/- (-)				+(6)

25 As can be seen in Table 15, the HCV Core-ISCOM vaccine produced no CTL positive responses in any of the 4 immunized macaques after the second or third immunizations. No positive CD8 γ -interferon and/or TNF- α intracellular staining was also observed, although backgrounds were high in these particular arrays. At least two of four macaques produced a strong LPA response after the second 30 immunizations, but only one remained positive after the third immunization. Two of four macaques produced positive CD4 intracellular staining after the second immunization and four of four after the third immunization.

As shown in Table 16, the HCV NS345Core₁₂₁-ISCOM vaccine after the second immunization produced CTL positive responses to peptide pools representing two or more HCV proteins in three of four macaques (two of these macaques had responses to peptide pools from NS3, NS5a and NS5b, one to peptide pools from core and NS3). CD8 positive γ -interferon and/or TNF- α intracellular staining to peptide pools representing two or more HCV proteins was positive in at least three of four macaques. One of four macaques produced a strong LPA response. At least three of four macaques produced CD4 positive intracellular staining to two or more HCV proteins. After the third immunization, only one of four macaques had a positive CTL response, CD8 positive intracellular staining and C04 positive intracellular staining. One other macaque had a positive LPA response and weak CD8+ CD4 intracellular staining. This decline in immunogenicity was likely due to instability of the vaccine formulation (see below).

As shown in Table 17, the HCV Core-ISCOM + NS5b-ISCOM vaccine produced a CTL positive response to NS5b in one of the 4 immunized macaques after the second immunization which did not remain positive after the third immunizaton. CD8 positive intracellular positive staining was observed in one of four animals post second. Two of four macaques produced a strong LPA response after the second immunization which did not remain positive after the third immunization. Two other macaques did develop a strong LPA response after the third immunization. Three or four developed positive CD4 intracellular staining. One developed positive CD8 intracellular staining.

Three weeks after the third immunization, it was noted that the physical appearance of the polyprotein vaccine solution was visibly turbid. The core vaccine also was turbid but less so. The Core-NS5 vaccine was also slightly turbid. Analysis of this turbidity in the polyprotein formulation indicated that the ISCOM particles had precipitated into large aggregates. These aggregates could be dispersed by vortexing with 0.1% TWEEN 80 detergent. It is probable that this change in the formulation of the vaccine occurred before the last immunization. This observed change in appearance of the vaccines may have affected their immunogenicity as cellular immune results declined in all three vaccines.

The immunogenicity of HCV Core-ISCOMS, NS345Core₁₂₁-ISCOMS and Core-ISCOMS + NS5b-ISCOMS as assessed by EIA antibody response is shown in Table 18. As can be seen, all three vaccines produced an antibody response by the third immunization to their corresponding HCV proteins, except for the 5 NS345Core₁₂₁-ISCOM vaccine. The NS345Core₁₂₁-ISCOM vaccine produced antibody responses to NS3, NS4 and a very strong antibody response to NS5, but no antibody response to HCV core.

Table 18. The immunogenicity of HCV Core-ISCOMS, NS345Core₁₂₁-ISCOMS, Core-ISOCMS + NS5b-ISCOMS vaccine two weeks post 2nd immunization and post 3rd immunization as assessed by EIA antibody response to HCV proteins.

Vaccine Macaque #	Anti-Core EIA Antibody Titer		Anti-NS3 EIA Antibody Titer		Anti-NS4 EIA Antibody Titer		Anti-NS5 EIA Antibody Titer	
	Post 2 nd	Post 3 rd	Post 2 nd	Post 3 rd	Post 2 nd	Post 3 rd	Post 2 nd	Post 3 rd
Core- ISCOM								
X020	66	226						
N001	87	46						
N086	363	396						
X010	108	137						
NS345 Core121/ ISCOM								
X016	<10	<10	<10	554	56	68	3,590	3,405
X008	<10	<10	66	995	14	44	2,109	3,213
X021	<10	<10	128	6,330	41	204	7,213	8,083
X023	<10	<10	<10	3,910	64	64	1,243	4,704
Core- ISCOM + NS5b- ISCOM								
X022	<10	18					<10	134
X014	<10	13					<10	693
N154	542	554					<10	272
N173	28	78					<10	258

EXAMPLE 17*Immunization of Chimpanzees with Recombinant HCV Protein and DNA**Vaccines*

Five groups of five chimps each were immunized IM at 0, 0.7, 2 and 5
5 months with the formulations presented below. Blood was collected at week 0, two weeks
subsequent to the second immunization, two weeks following the third immunization
and two weeks after the fourth immunization.

Formulation 1: 20 µg E1E2 polypeptide + MF59 + 500 µg CpG (produced as
10 described above);

Formulation 2: 1 mg NS345Core₁₂₁-ISCOM (produced as described above);

Formulation 3: 6 mg each of CTAB-PLG-E1E2 (bp 574-2427, encoding
15 amino acids 192-809 of the HCV polyprotein, numbered relative to HCV-1); CTAB-
PLG-NS34a (bp 3079-5133, encoding amino acids 1027-1711 of the HCV
polyprotein, numbered relative to HCV-1); CTAB-PLG-NS34ab (bp 4972-5916,
encoding amino acids 1658-1972 of the HCV polyprotein, numbered relative to
HCV-1); CTAB-PLG-NS5a (bp 5917-7260, encoding amino acids 1973-2420 of the
HCV polyprotein, numbered relative to HCV-1);

Formulation 4: 6 mg each of E1E2 DNA, NS34a DNA, NS34ab DNA and
20 NS5a DNA, having the same coordinates as described above, delivered without PLG
via electroporation (see, e.g., U.S. Patent Nos. 6,132,419; 6,451,002, 6,418,341,
6233,483, U.S. Patent Publication No. 2002/0146831; and International Publication
No. WO/0045823, for this delivery technique). Results are shown in Figures 8-10.

As can be seen, in Figure 8, all vaccines were capable of priming CD4+ and
25 CD8+ cells specific to HCV. Thus, all vaccines were successful at inducing a T cell
response to HCV. Determination of the results for the PLG-DNA from formulation 3
at two weeks subsequent to the fourth vaccination is in progress.

As shown in Figures 9 and 10, multiple T cell specificities were induced by
the two vaccines. Both vaccines primed T-cells specific for multiple T cell epitopes.

30 As can be seen in Tables 19 and 20, E1E2 adjuvanted with MF59 primed

anti-E1E2 titers. CpG enhanced anti-E1E2 responses as well as TH1 responses and the ISCOM and the two DNA vaccines were capable of priming CD4+ and CD8+ T cell responses to HCV.

Table 19. Anti-E1E2 EIA antibody titers in chimps immunized with Electroporated DNA E1E2NS345a or PLG DNA E1E2NS345

Vaccine	Chimp	Pre 1 st	Post 2 nd	Post 3 rd	Post 4 th
Electroporated DNA	4X0330	-	-	-	9
E1E2-NS34A-	4X0335	-	-	-	10
NS4AB-NS5A ^a	4X0348 ^c	10	457	198	50
	4X0354 ^d	206	1,261	1,197	207
	4X0368 ^c	245	1,426	1,267	358
PLG DNA	4X0238	-	-	30	10
E1E2-NS34A-	4X0239	-	104	309	-
NS4AB-NS5A ^b	4X0250	-	-	12	-
	4X0278	-	-	29	-
	4X0288	-	-	12	-

^aElectroporated IM with 1.5 mg of each plasmid at 0, 0.7, 2 and 5 months.

Bleeds were taken 14 days after each immunization.

^bIM immunization with 1.5 mg of each PLG plasmid at 0, 0.7, 2 and 6 months.

Bleeds were taken 14 days after each immunization.

^cPrior E2 immunization

^dPrior E1E2 immunization

Table 20. Immunogenicity in chimps of low dose (20 µg) HCV E1E2 antigen using MF59 or MF59 combined with CpG as adjuvants (2 wks post 3rd)

Vaccine ^a	Chimp	E1E2EIA Ab Titer	E1E2 Ab GMT	CD4+ (ICS)
E1E2/ MF59	4x0419	84	-	-
	4x0420	101	-	-
	4x0431	131	261	-
	4x0371	421	-	-
	4x0372	2,580	+/-	-
			P = 0.029 ^b	
E1E2/ MF59/CpG	4x0410	8,835	-	-
	4x0426	2,713	-	-
	4x0365	3,201	2,713	+
	4x0367	510	-	-
	4x0346	1,238	++	-

^aChimps immunized IM at 0, 1 and 6 mos with 20 µg of E1E2 antigen using MF59 with or without 500 µg of CpG. Serum samples were obtained 14 days after last immunization.

^bChimps immunized with E1E2 using CpG combined with MF59 as adjuvant produced significantly higher (P<0.05) levels of E1E2 EIA antibody than chimpanzees with E1E2 using MF59 alone.*

Thus, HCV polypeptides and polynucleotides, either alone or as fusions, to stimulate cell-mediated immune responses, are disclosed. Although preferred embodiments of the subject invention have been described in some detail, it is understood that obvious variations can be made without departing from the spirit and 5 the scope of the invention as defined by the appended claims.

We claim:

1. A fusion protein comprising HCV polypeptides, wherein the HCV polypeptides consist essentially of an NS3, an NS4, an NS5 and a core polypeptide of a hepatitis C virus (HCV), wherein said core polypeptide consists of amino acids 1-121 of the HCV polyprotein, numbered relative to the full-length HCV-1 polyprotein.
2. The fusion protein of claim 1, wherein the NS5 polypeptide is an NS5a polypeptide.
3. The fusion protein of claim 1, wherein the NS5 polypeptide is an NS5b polypeptide.
4. The fusion protein of claim 1, wherein the NS5 polypeptide is an NS5a and an NS5b polypeptide.
- 20 6. The fusion protein of claim 4, wherein the protein comprises the sequence of amino acids of SEQ ID NO:8.
- 25 7. A fusion protein according to any of claims 1-6, wherein at least one of the HCV polypeptides is derived from a different strain of HCV than the other HCV polypeptides.
- 30 8. A composition comprising:
 - (a) a fusion protein according to any of claims 1-7; and
 - (b) a pharmaceutically acceptable excipient.

9. The composition of claim 8, further comprising an adjuvant.

10. The composition of claim 8, further comprising a CpG oligonucleotide.

5

11. The composition of claim 8, wherein said fusion protein is adsorbed to or

entrapped within a microparticle or ISCOM.

10 12. The composition of claim 8, further comprising a polynucleotide encoding an HCV E1E2 complex.

13. An isolated and purified polynucleotide that encodes a fusion protein according to any of claims 1-7.

15

14. A composition comprising:

- (a) the isolated and purified polynucleotide of claim 13; and
- (b) a pharmaceutically acceptable excipient.

20 15. The composition of claim 14, further comprising an adjuvant.

16. The composition of claim 14, wherein said polynucleotide is adsorbed to

or entrapped within a microparticle.

25

17. The composition of claim 14, further comprising a polynucleotide encoding an HCV E1E2 complex.

30 18. A method of activating T cells of a vertebrate subject which recognize an epitope of an HCV polypeptide, comprising the step of:

administering the composition of any of claims 8-12 to said vertebrate

subject, whereby a population of activated T cells recognizes an epitope of the NS3, NS4, NS5 and/or core polypeptides.

19. A method of activating T cells of a vertebrate subject which recognize
5 an epitope of an HCV polypeptide, comprising the step of:

administering the composition of any of claims 14-17 to said vertebrate
subject, whereby a population of activated T cells recognizes an epitope of the NS3,
NS4, NS5 and/or core polypeptides.

10 20. The method of claim 19, wherein the polynucleotide is administered
via
electroporation.

15 21. Use of a composition according to any of claims 8-12 and 14-17 for
activating T cells of a vertebrate subject which recognize an epitope of an HCV
polypeptide, wherein a population of activated T cells recognizes an epitope of the
NS3, NS4, NS5 and/or core polypeptides.

20 22. Use of a fusion protein according to any of claims 1-7 for the
manufacture of a medicament for activating T cells of a vertebrate subject which
recognize an epitope of an HCV polypeptide, wherein a population of activated T
cells recognizes an epitope of the NS3, NS4, NS5 and/or core polypeptides.

25 23. Use of a polynucleotide according to claim 13 for the manufacture of
a
medicament for activating T cells of a vertebrate subject which recognize an epitope
of an HCV polypeptide, wherein a population of activated T cells recognizes an
epitope of the NS3, NS4, NS5 and/or core polypeptides.

1/21

HCV Genome and Recombinant Proteins

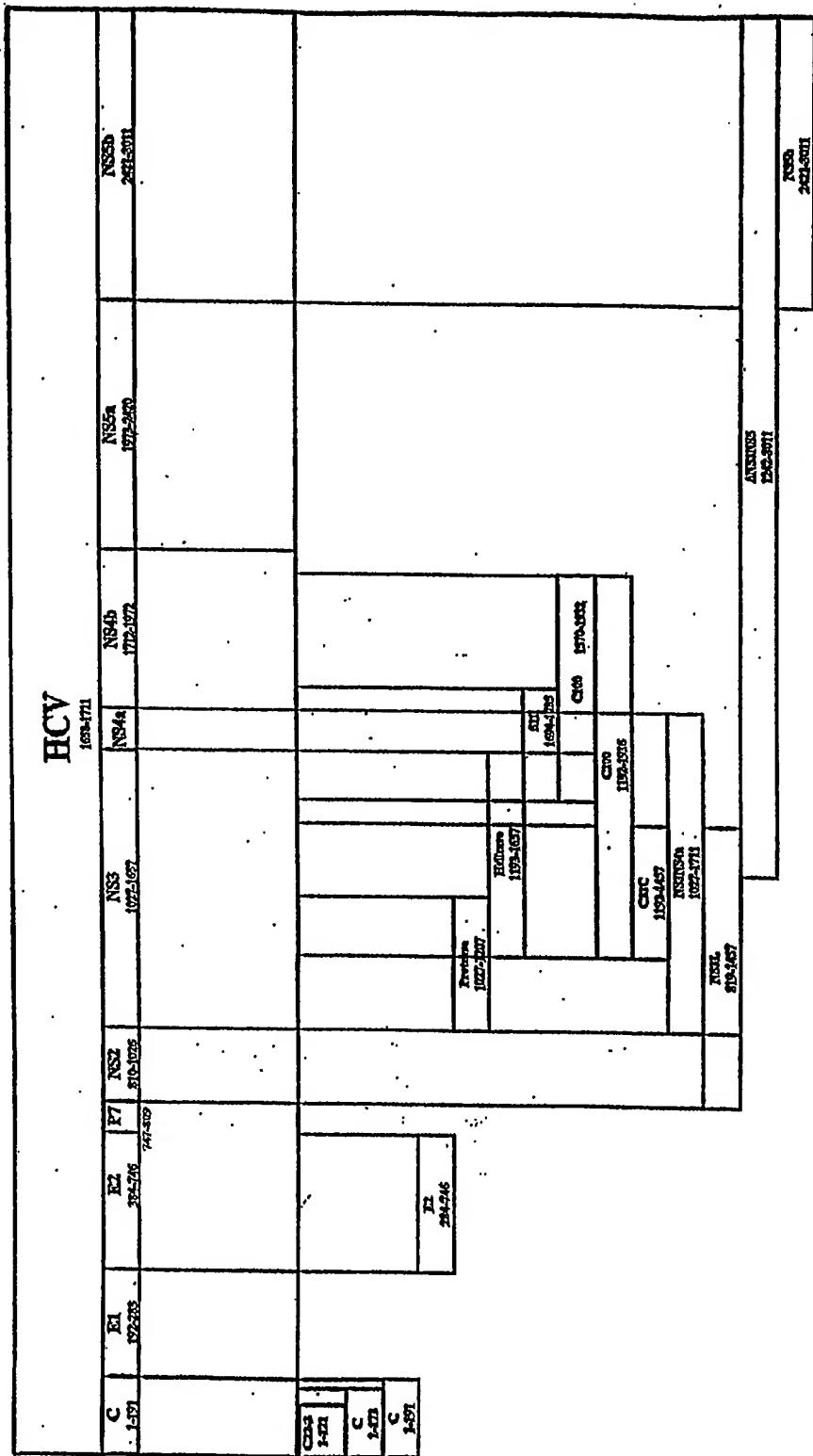


FIG. 1

2/21

1 M A P I T A Y A Q Q
 ATG GCG CCC ATC ACG GCG TAC GCC CAG CAG
 10

T R G L L G C I I T S L T G R
 ACA AGG GGC CTC CTA GGG TGC ATA ATC ACC AGC CTA ACT GGC CGG
 20

D K N Q V E G E V Q I V S T A
 GAC AAA AAC CAA GTG GAG GGT GAG GTC CAG ATT GTG TCA ACT GCT
 30

A Q T F L A T C I N G V C W T
 GCC CAA ACC TTC CTG GCA ACG TGC ATC AAT GGG GTG TGC TGG ACT
 40

V Y H G A G T R T I A S P K G
 GTC TAC CAC GGG GCC GGA ACG AGG ACC ATC GCG TCA CCC AAG GGT
 50

P V I Q M Y T N V D Q D L V G
 CCT GTC ATC CAG ATG TAT ACC AAT GTA GAC CAA GAC CTT GTG GGC
 60

W P A P Q G S R S L T P C T C
 TGG CCC GCT CCG CAA GGT AGC CGA TCA TTG ACA CCC TGC ACT TGC
 70

G S S D L Y L V T R H A D V I
 GGC TCC TCG GAC CTT TAC CTG GTC ACG AGG CAC GCC GAT GTC ATT
 80

P V R R R G D S R G S L L S P
 CCC GTG CGC CGG CGG GGT GAT AGC AGG GGC AGC CTG CTG TCG CCC
 90

R P I S Y L K G S S G G P L L
 CGG CCC ATT TCC TAC TTG AAA GGC TCC TCG GGG GGT CCG CTG TTG
 100

C P A G H A V G I F R A A V C
 TGC CCC GCG GGG CAC GCC GTG GGC ATA TTT AGG GCC GCG GTG TGC
 110

T R G V A K A V D F I P V E N
 ACC CGT GGA GTG GCT AAG GCG GTG GAC TTT ATC CCT GTG GAG AAC
 120

L E T T M R S
 CTA GAG ACA ACC ATG AGG TCC
 130

140

150

160

170

180

FIG. 2

3/21

MATURE E1

SerPheSerIlePheLeuLeuAlaLeuLeuSerCysLeuThrValProAlaSerAlaTyr 192
 TCTTTCTCTATCTCCCTCTGGCCCTGCTCTGCTTGACTGTGCCGCTTCGGCCTAC
 AGAAAGAGATAGAAGGAAGACCGGGACGAGAGAACGAACTGACACGGCGAAGCCGGATG

GlnValArgAsnSerThrGlyLeuTyrHisValThrAsnAspCysProAsnSerSerIle 212
 CAAAGTGGCAACTCCACGGGCTCTACCACGTCAACATGATTGCCCTAACTCGAGTATT
 GTTCACGGTTGAGGTGCCCGAGATGGTCAGTGGTTACTAACGGGATTGAGCTCATAA

ValTyrGluAlaAlaAspAlaIleLeuHisThrProGlyCysValProCysValArgGlu 232
 GTGTACGAGGCGGCCGATGCCATCCTGCACACTCCGGGTGCGTCCCTTGCCTCGAG
 CACATGCTCCGCCGGTACGGTAGGACGTGTGAGGCCACCGCAGGGAACGCAAGCGCTC

GlyAsnAlaSerArgCysTrpValAlaMetThrProThrValAlaThrArgAspGlyLys 252
 GGCAACGCCCTCGAGGTGTTGGGTGGCGATGACCCCTACGGTGGCCACCAGGGATGGCAAA
 CGTTGCGGAGCTCCACAACCCACCGTACTGGGATGCCACCGGTGGTCCCTACCGTTT

LeuProAlaThrGlnLeuArgArgHisIleAspLeuLeuValGlySerAlaThrLeuCys 272
 CTCCCCGCGACGCAGCTCGACGTCACATCGATCTGCTTGTGCGGAGCGCCACCCCTCTGT
 GAGGGGCGCTGCGTCAAGCTGCAAGCTGAGTAGCTAGACGAACAGCCCTCGCGGTGGGAGACA

SerAlaLeuTyrValGlyAspLeuCysGlySerValPheLeuValGlyGlnLeuPheThr 292
 TCGGCCCTCTACGTGGGGGACCTGTGCGGGCTGTCTTGTGCGGCCACTGTTTACC
 AGCCGGGAGATGCACCCCTGGACACGCCAGACAGAAAGAACAGCCGGTTGACAATGG

PheSerProArgArgHisTrpThrThrGlnGlyCysAsnCysSerIleTyrProGlyHis 312
 TTCTCTCCCAGGCGCCACTGGACGACGCAAGGTTGCAATTGCTCTATCTATCCGGCCAT
 AAGAGAGGGTCCGCGGTGACCTGCTGCCAACGTTAACGAGATAGATAGGGCCGGTA

IleThrGlyHisArgMetAlaTrpAspMetMetAsnTrpSerProThrThrAlaLeu 332
 ATAACGGGTCAACCGCATGGCATGGATATGATGATGAACTGGTCCCTACGACGGCGTG
 TATTGCCAGTGGCGTACCGTACCCCTACTACTACTTGTGACCAGGGATGCTGCCGCAAC

ValMetAlaGlnLeuLeuArgIleProGlnAlaIleLeuAspMetIleAlaGlyAlaHis 352
 GTAAATGGCTCAGCTGCTCCGGATCCCACAAGCCATCTGGACATGATCGCTGGTGTGTCAC
 CATTACCGAGTCGACGAGGCCTAGGGTGTCTGGTAGAACCTGTACTAGCGACCACGAGTG

TrpGlyValLeuAlaGlyIleAlaTyrPheSerMetValGlyAsnTrpAlaLysValLeu 372
 TGGGGAGTCCTGGGGCATAGCGTATTCCTCATGGGGAACTGGCGAAGGTGCTG
 ACCCCTCAGGACCGCCCGTATCGCATAAAGAGGTACCAACCCCTGACCGCTTCCAGGAC

E2

ValValLeuLeuLeuPheAlaGlyValAspAlaGluThrHisValThrGlyGlySerAla 392
 GTAGTGCTGCTGCTATTGCGCGTCGACCGGGAAACCCACGTCACCGGGGGAAAGTGCC
 CATCACGACGACGATAAACGGCGCAGCTGCCCTTGGGTGCACTGGCCCCCTCACGG

GlyHisThrValSerGlyPheValSerLeuLeuAlaProGlyAlaLysGlnAsnValGln 412
 GGCCACACTGTGTGGATTGTTAGCCTCTCGCACCAGGCGCAAGCAGAACGTCCAG
 CGGGTGTGACACAGACCTAAACAATCGGAGGAGCGTGGTCCGCGGTTCGTCTGCAGGTC

FIG. 3A

4/21

LeuIleAsnThrAsnGlySerTrpHisLeuAsnSerThrAlaLeuAsnCysAsnAspSer 432
 CTGATCAACACCAACGGCAGTTGGCACCTCAATAGCACGGCCCTGAAGTCAATGATAGC
 GACTAGTTGTGGTTGCCGTCAACCGTGGAGTTATCGTGCCGGACTTGACGTTACTATCG

LeuAsnThrGlyTrpLeuAlaGlyLeuPheTyrHisHisLysPheAsnSerSerGlyCys 452
 CTCAACACCGGCTGGTGGCAGGGCTTTCTATCACCAAGTCAACTCTCAGGCTGT
 GAGTTGTGGCCGACCAACCGTCCCAGAAAGATAGTGGTGTCAAGTTGAGAAGTCCGACA

ProGluArgLeuAlaSerCysArgProLeuThrAspPheAspGlnGlyTrpGlyProIle 472
 CCTGAGAGGGCTAGCCAGCTGCCGACCCCTTACCGATTGGTACCGAGGGCTGGGGCCCTATC
 GGACTCTCCGATCGGTGACGGCTGGGAATGGCTAAACACTGGTCCCACCCGGGATAG

SerTyrAlaAsnGlySerGlyProAspGlnArgProTyrCysTrpHisTyrProProLys 492
 AGTTATGCCAACCGAACGGGCCCCGACCAGCAGCCCTACTGCTGGCAGTACCCCCCAAAA
 TCAATACGGTTGCCCTCGCCGGGCTGGTCGCGGGATGACGACCGTGTGGGGGTTT

ProCysGlyIleValProAlaLysSerValCysGlyProValTyrCysPheThrProSer 512
 CCTTGCCTGATTGTGCCCGCGAACAGTGTGTGGTCCGGTATATTGCTTCACCTCCCAGC
 GGAACGCCATAACACGGCGCTCTCACACACACCAGGCCATATAACGAAGTGAGGGTCG

ProValValValGlyThrThrAspArgSerGlyAlaProThrTyrSerTrpGlyGluAsn 532
 CCCGTGGTGGTGGGAACGACCGACAGGTGCGGCTGGCTGTCCAGCCCCGGGGTGGATGTCGACCCACTTTA
 GGGCACCACCAACCTTGCTGGCTGTCCAGCCCCGGGGTGGATGTCGACCCACTTTA

AspThrAspValPheValLeuAsnAsnThrArgProProLeuGlyAsnTrpPheGlyCys 552
 GATACGGACGTCTCGTCTTAACAAATACCAGGCCACCGCTGGCAATTGGTTCGGTTGT
 CTATGCCCTGAGAACAGGAATTGTTATGGTCCGGTGGCACCCTTAACCAAGCCAACA

ThrTrpMetAsnSerThrGlyPheThrLysValCysGlyAlaProProCysValIleGly 572
 ACCTGGATGAACTCAACTGGATTCAACAAAGTGTGGAGCGCCCTCCCTGTGTCACTCGGA
 TGGACCTACTTGAGTTGACCTAACAGGGTTACAGGGTGTGGGGGTTGACTAACGAAGGCGTGTAGGCCTGCGG

GlyAlaGlyAsnAsnThrLeuHisCysProThrAspCysPheArgLysHisProAspAla 592
 GGGGCGGGCAACAAACACCCCTGCACTGCCCACTGATTGCTTCCGCAAGCATTCCGGACGCC
 CCCCGCCCGTGTGGTGGACGTGACGGGGTGAACGAAAGGCGTGTAGGCCTGCGG

ThrTyrSerArgCysGlySerGlyProTrpIleThrProArgCysLeuValAspTyrPro 612
 ACATACTCTCGGTGCCGCTCCGGTCCCTGGATCACACCCAGGTGCCTGGTCGACTACCCG
 TGATGAGAGGCCACGCCAGGGCAGGGACCTAGTGTGGTCCACGGACCAGCTGATGGGC

TyrArgLeuTrpHisTyrProCysThrIleAsnTyrThrIlePheLysIleArgMetTyr 632
 TATAGGCTTGGCATTATCCTTGTACCATCAACTACACTATATTAAAATCAGGATGTAC
 ATATCCGAAACCGTAATAGGAACATGGTAGTTGATGTGATATAAAATTAGTCCTACATG

ValGlyGlyValGluHisArgLeuGluAlaAlaCysAsnTrpThrArgGlyGluArgCys 652
 GTGGGAGGGGTCGAGCACAGGCTGGAAGCTGCCCTGCAACTGGACGCGGGCGAACGTTGC
 CACCCCTCCCCAGCTCGTGTCCGACCTCGACGGACGTTGACCTGCGCCCCGTTGCAACG

AspLeuGluAspArgAspArgSerGluLeuSerProLeuLeuLeuThrThrThrGlnTrp 672
 GATCTGGAAGATAGGGACAGGTCCGAGCTCAGCCCGTTACTGCTGACCAACTACACAGTGG
 CTAGACCTCTATCCCTGTCCAGGCTCGAGTCGGGCAATGACGACTGGTGTGTCACC

FIG. 3B

5/21

GlnValLeuProCysSerPheThrThrLeuProAlaLeuSerThrGlyLeuIleHisLeu 692
 CAGGTCCCTCCCGTGTCCCTTCACAACCCCTGCCAGCCCTGTCACCGGCCATCCACCTC
 GTCCAGGAGGGACAAGGAAGTGTGGGACGGTCGGAACAGGTGGCCGGAGTAGGTGGAG

HisGlnAsnIleValAspValGlnTyrLeuTyrGlyValGlySerSerIleAlaSerTrp 712
 CACCAGAACATTGTGGACGTGCAGTACTTGTACGGGTGGGTCAAGCATCGCGTCCCTGG
 GTGGTCTTGTAAACACCTGCACGTCATGAACATGCCACCCAGTCGTAGCGCAGGACC

AlaIleLysTrpGluTyrValValLeuLeuLeuPheLeuLeuAlaAspAlaArgValCys 732
 GCCATTAAGTGGGACTACGTCGTCCCTGTCCTGCTGCAGACGCCGCGCTCTGC
 CGGTAAATTCAACCTCATGCAGCAGGACAAGGAAGACGAACGTCTGCAGCAGACG

P7
 SerCysLeuTrpMetMetLeuLeuIleSerGlnAlaGluAlaAlaLeuGluAsnLeuVal 752
 TCCCTGCTTGTGGATGATGCTACTCATATCCCAAGCGGAAGCGGCTTGGAGAACCTCGTA
 AGGACGAACACCTACTACGATGAGTATAGGGTTCGCCCTGCCAACCTCTGGAGCAT

IleLeuAsnAlaAlaSerLeuAlaGlyThrHisGlyLeuValSerPheLeuValPhePhe 772
 ATACTTAATGCAGCATCCCTGGCCGGACGCACGGTCTTGTATCCTCCTCGTGTCTTC
 TATGAATTACGTCGTAGGGACCGGCCCTGCGTGCAGAACATAGGAAGGAGCACAGAAG

CysPheAlaTrpTyrLeuLysGlyLysTrpValProGlyAlaValTyrThrPheTyrGly 792
 TGCTTTGCATGGTATCTGAAGGGTAAGTGGGTGCCGGAGCAGGTCTACACCTTCTACGGG
 ACGAAACGTACCATAGACTTCCCATTCAACCCACGGCCTGCCAGATGTGGAAGATGCC

MetTrpProLeuLeuLeuLeuLeuAlaLeuProGlnArgAlaTyrAlaOC 809
 ATGTGGCCTCTCCTCGCTCTGCTTGGCGTGTGCCAGCGGGCGTACCGCTAA
 TACACCGGAGAGGAGGGACGAGGACAACCGAACGGGTGCCGCATGCGCATT

FIG. 3C

6/21

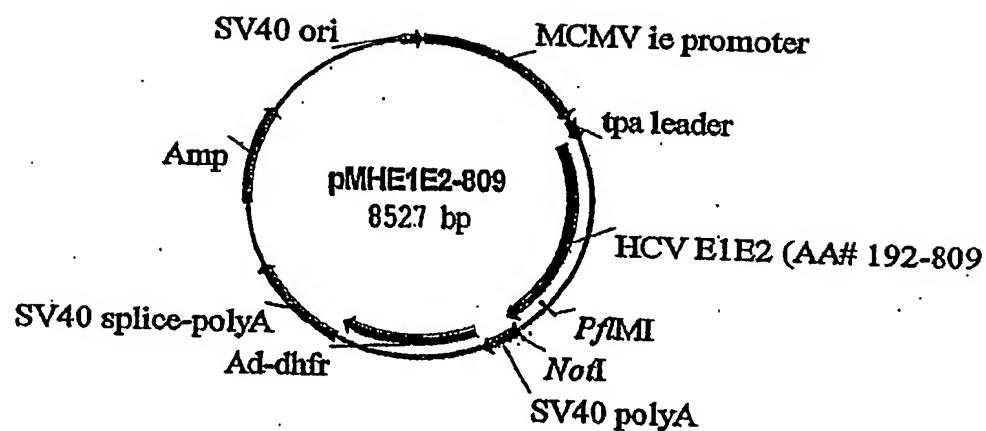


FIG. 4

7/21

M A A Y A A Q G Y K
 ATG GCT GCA TAT GCA GCT CAG GGC TAT AAG

V L V L N P S V A A T L G F G
 20
 GTG CTA GTA CTC AAC CCC TCT GTT GCT GCA ACA CTG GGC TTT GGT

A Y M S K A H G I D P N I R T
 30
 GCT TAC ATG TCC AAG GCT CAT GGG ATC GAT CCT AAC ATC AGG ACC

G V R T I T T G S P I T Y S T
 40
 GGG GTG AGA ACA ATT ACC ACT GGC AGC CCC ATC ACG TAC TCC ACC

Y G K F L A D G G C S G G A Y
 50
 TAC GGC AAG TTC CTT GCC GAC GGC GGG TGC TCG GGG GGC GCT TAT

D I I C D E C H S T D A T S
 60
 GAC ATA ATA ATT TGT GAC GAG TGC CAC TCC ACG GAT GCC ACA TCC

I L G I G T V L D Q A E T A G
 70
 90 ATC TTG GGC ATT GGC ACT GTC CTT GAC CAA GCA GAG ACT GCG GGG

A R L V V L A T A T P P G S V
 100
 GCG AGA CTG GTT GTG CTC GCC ACC GCC ACC CCT CCG GGC TCC GTC

T V P H P N I E E V A L S T T
 110
 ACT GTG CCC CAT CCC AAC ATC GAG GAG GTT GCT CTG TCC ACC ACC

G E I P F Y G K A I P L E V I
 120
 GGA GAG ATC CCT TTT TAC GGC AAG GCT ATC CCC CTC GAA GTA ATC

K G G R H L I F C H S K K K C
 130
 150 AAG GGG AGA CAT CTC ATC TTC TGT CAT TCA AAG AAG AAG TGC

D E L A A K L V A L G I N A V
 160
 GAC GAA CTC GCC GCA AAG CTG GTC GCA TTG GGC ATC AAT GCC GTG

A Y Y R G L D V S V I P T S G
 170
 180 GCC TAC TAC CGC GGT CTT GAC GTG TCC GTC ATC CCG ACC AGC GGC

FIG. 5A

8/21

200

D V V V V A T D A L M T G Y T
GAT GTT GTC GTC GTG GCA ACC GAT GCC CTC ATG ACC GGC TAT ACC

210 220

G D F D S V I D C N T C V T Q
GGC GAC TTC GAC TCG GTG ATA GAC TGC AAT ACG TGT GTC ACC CAG

230

T V D F S L D P T F T I E T I
ACA GTC GAT TTC AGC CTT GAC CCT ACC TTC ACC ATT GAG ACA ATC

240 250

T L P Q D A V S R T Q R R G R
ACG CTC CCC CAA GAT GCT GTC TCC CGC ACT CAA CGT CGG GGC AGG

260

T G R G K P G I Y R F V A P G
ACT GGC AGG GGG AAG CCA GGC ATC TAC AGA TTT GTG GCA CCG GGG

270 280

E R P S G M F D S S V L C E C
GAG CGC CCC TCC GGC ATG TTC GAC TCG TCC GTC CTC TGT GAG TGC

290

Y D A G C A W Y E L T P A E T
TAT GAC GCA GGC TGT GCT TGG TAT GAG CTC ACG CCC GCC GAG ACT

300 310

T V R L R A Y M N T P G L P V
ACA GTT AGG CTA CGA GCG TAC ATG AAC ACC CCG GGG CTT CCC GTG

320

C Q D H L E F W E G V F T G L
TGC CAG GAC CAT CTT GAA TTT TGG GAG GGC GTC TTT ACA GGC CTC

330 340

T H I D A H F L S Q T K Q S G
ACT CAT ATA GAT GCC CAC TTT CTA TCC CAG ACA AAG CAG AGT GGG

350

E N L P Y L V A Y Q A T V C A
GAG AAC CTT CCT TAC CTG GTA GCG TAC CAA GCC ACC GTG TGC GCT

360 370

R A Q A P P S W D Q M W K C
AGG GCT CAA GCC CCT CCC CCA TCG TGG GAC CAG ATG TGG AAG TGT

380

L I R L K P T L H G P T P L L
TTG ATT CGC CTC AAG CCC ACC CTC CAT GGG CCA ACA CCC CTG CTA

FIG. 5B

9/21

390 400
 Y R L G A V Q N E I T L T H P
 TAC AGA CTG GGC GCT GTT CAG AAT GAA ATC ACC ACC CTG ACG CAC CCA
 410
 V T K Y I M T C M S A D L E V
 GTC ACC AAA TAC ATC ATG ACA TGC ATG TCG GCC GAC CTG GAG GTC
 420 430
 V T S T W V L V G G V L A A L
 GTC ACG AGC ACC TGG GTG CTC GTT GGC GGC GTC CTG GCT GCT TTG
 440
 A A Y C L S T G C V V I V G R
 GCC GCG TAT TGC CTG TCA ACA GGC TGC GTG GTC ATA GTG GGC AGG
 450 460
 V V L S G K P A I I P D R E V
 GTC GTC TTG TCC GGG AAG CCG GCA ATC ATA CCT GAC AGG GAA GTC
 470
 L Y R E F D E M E E C S Q H L
 CTC TAC CGA GAG TTC GAT GAG ATG GAA GAG TGC TCT CAG CAC TTA
 480 490
 P Y I E Q G M M L A E Q F K Q
 CCG TAC ATC GAG CAA GGG ATG ATG CTC GCC GAG CAG TTC AAG CAG
 500
 K A L G L L Q T A S R Q A E V
 AAG GCC CTC GGC CTC CTG CAG ACC GCG TCC CGT CAG GCA GAG GTT
 510 520
 I A P A V Q T N W Q K L E T F
 ATC GCC CCT GCT GTC CAG ACC AAC TGG CAA AAA CTC GAG ACC TTC
 530
 W A K H M W N F I S G I Q Y L
 TGG GCG AAG CAT ATG TGG AAC TTC ATC AGT GGG ATA CAA TAC TTG
 540 550
 A G L S T L P G N P A I A S L
 GCG GGC TTG TCA ACG CTG CCT GGT AAC CCC GCC ATT GCT TCA TTG
 560
 M A F T A A V T S P L T T S Q
 ATG GCT TTT ACA GCT GCT GTC ACC AGC CCA CTA ACC ACT AGC CAA
 570 580
 T L L F N I L G G W V A A Q L
 ACC CTC CTC TTC AAC ATA TTG GGG GGG TGG GTG GCT GCC CAG CTC

FIG. 5C

10/21

590

A A P G A A T A F V G A G L A
GCC GCC CCC GGT GCC GCT ACT GCC TTT GTG GGC GCT GGC TTA GCT

600

G A A I G S V G L G K V L I D
GGC GCC GCC ATC GGC AGT GTT GGA CTG GGG AAG GTC CTC ATA GAC

620

I L A G Y G A G V A G A L V A
ATC CTT GCA GGG TAT GGC GCG GGC GTG GCG GGA GCT CTT GTG GCA

630

F K I M S G E V P S T E D L V
TTC AAG ATC ATG AGC GGT GAG GTC CCC TCC ACG GAG GAC CTG GTC

640

N L L P A I L S P G A L V V G
AAT CTA CTG CCC GCC ATC CTC TCG CCC GGA GCC CTC GTA GTC GGC

650

V V C A A I L R R H V G P G E
GTG GTC TGT GCA GCA ATA CTG CGC CGG CAC GTT GGC CCG GGC GAG

660

G A V Q W M N R L I A F A S R
GGG GCA GTG CAG TGG ATG AAC CGG CTG ATA GCC TTC GCC TCC CGG

680

G N H V S P T H Y V P E S D A
GGG AAC CAT GTT TCC CCC ACG CAC TAC GTG CCG GAG AGC GAT GCA

690

A A R V T A I L S S L T V T Q
GCT GCC CGC GTC ACT GCC ATA CTC AGC AGC CTC ACT GTA ACC CAG

700

L L R R L H Q W I S S E C T T
CTC CTG AGG CGA CTG CAC CAG TGG ATA AGC TCG GAG TGT ACC ACT

710

P C S G S W L R D I W D W I C
CCA TGC TCC GGT TCC TGG CTA AGG GAC ATC TGG GAC TGG ATA TGC

720

E V L S D F K T W L K A K L M
GAG GTG TTG AGC GAC TTT AAG ACC TGG CTA AAA GCT AAG CTC ATG

730

P Q L P G I P F V S C Q R G Y
CCA CAG CTG CCT GGG ATC CCC TTT GTG TCC TGC CAG CGC GGG TAT

740

750

760

770

FIG. 5D

11/21

780
 K G V W R G D G I M H T R C H
 AAG GGG GTC TGG CGA GGG GAC GGC ATC ATG CAC ACT CGC TGC CAC

 800
 C G A E I T G H V K N G T M R
 TGT GGA GCT GAG ATC ACT GGA CAT GTC AAA AAC GGG ACG ATG AGG

 810
 I V G P R T C R N M W S G T F
 ATC GTC GGT CCT AGG ACC TGC AGG AAC ATG TGG AGT GGG ACC TTC

 830
 P I N A Y T T G P C T P L P A
 CCC ATT AAT GCC TAC ACC ACG GGC CCC TGT ACC CCC CTT CCT GCG

 840
 P N Y T F A L W R V S A E E Y
 CCG AAC TAC ACG TTC GCG CTA TGG AGG GTG TCT GCA GAG GAA TAC

 860
 V E I R Q V G D F H Y V T G M
 GTG GAG ATA AGG CAG GTG GGG GAC TTC CAC TAC GTG ACG GGT ATG

 870
 T T D N L K C P C Q V P S P E
 ACT ACT GAC AAT CTT AAA TGC CCG TGC CAG GTC CCA TCG CCC GAA

 880
 F F T E L D G V R L H R F A P
 TTT TTC ACA GAA TTG GAC GGG GTG CGC CTA CAT AGG TTT GCG CCC

 900
 P C K P L L R E E V S F R V G
 CCC TGC AAG CCC TTG CTG CGG GAG GAG GTA TCA TTC AGA GTA GGA

 920
 L H E Y P V G S Q L P C E P E
 CTC CAC GAA TAC CCG GTA GGG TCG CAA TTA CCT TGC GAG CCC GAA

 930
 P D V A V L T S M L T D P S H
 CCG GAC GTG GCC GTG TTG ACG TCC ATG CTC ACT GAT CCC TCC CAT

 950
 I T A E A A G R R L A R G S P
 ATA ACA GCA GAG GCG GCC GGG CGA AGG TTG GCG AGG GGA TCA CCC

 960
 P S V A S S S A S Q L S A P S
 CCC TCT GTG GCC AGC TCC TCG GCT AGC CAG CTA TCC GCT CCA TCT

FIG. 5E

12/21

980
 L K A T C T A N H D S P D A E
 CTC AAG GCA ACT TGC ACC GCT AAC CAT GAC TCC CCT GAT GCT GAG

990 1000
 L I E A N L L W R Q E M G G N
 CTC ATA GAG GCC AAC CTC CTA TGG AGG CAG GAG ATG GGC GGC AAC

1010
 I T R V E S E N K V V I L D S
 ATC ACC AGG GTT GAG TCA GAA AAC AAA GTG GTG ATT CTG GAC TCC

1020 1030
 F D P L V A E E D E R E I S V
 TTC GAT CCG CTT GTG GCG GAG GAC GAG CGG GAG ATC TCC GTA

1040
 P A E I L R K S R R F A Q A L
 CCC GCA GAA ATC CTG CGG AAG TCT CGG AGA TTC GCC CAG GCC CTG

1050 1060
 P V W A R P D Y N P P L V E T
 CCC GTT TGG GCG CGG CCG GAC TAT AAC CCC CCG CTA GTG GAG ACG

1070
 W K K P D Y E P P V V H G C P
 TGG AAA AAG CCC GAC TAC GAA CCA CCT GTG GTC CAT GGC TGC CCG

1080 1090
 L P P P K S P P V P P P R K K
 CTT CCA CCT CCA AAG TCC CCT CCT GTG CCT CCG CCT CGG AAG AAG

1100
 R T V V L T E S T L S T A L A
 CGG ACG GTG GTC CTC ACT GAA TCA ACC CTA TCT ACT GCC TTG GCC

1110 1120
 E L A T R S F G S S S T S G I
 GAG CTC GCC ACC AGA AGC TTT GGC AGC TCC TCA ACT TCC GGC ATT

1130
 T G D N T T T S S E P A P S G
 ACG GGC GAC AAT ACG ACA ACA TCC TCT GAG CCC GCC CCT TCT GGC

1140 1150
 C P P D S D A E S Y S S M P P
 TGC CCC CCC GAC TCC GAC GCT GAG TCC TAT TCC TCC ATG CCC CCC

1160
 L E G E P G D P D L S D G S W
 CTG GAG GGG GAG CCT GGG GAT CCG GAT CTT AGC GAC GGG TCA TGG

FIG. 5F

13/21

1170 1180
 S T V S S E A N A E D V V C C
 TCA ACG GTC AGT AGT GAG GCC AAC GCG GAG GAT GTC GTG TGC TGC

1190
 S M S Y S W T G A L V T P C A
 TCA ATG TCT TAC TCT TGG ACA GGC GCA CTC GTC ACC CCG TGC GCC

1200 1210
 A E E Q K L P I N A L S N S L
 GCG GAA GAA CAG AAA CTG CCC ATC AAT GCA CTA AGC AAC TCG TTG

1220
 L R H H N L V Y S T T S R S A
 CTA CGT CAC CAC AAT TTG GTG TAT TCC ACC ACC TCA CGC AGT GCT

1230 1240
 C Q R Q K K V T F D R L Q V L
 TGC CAA AGG CAG AAG AAA GTC ACA TTT GAC AGA CTG CAA GTT CTG

1250
 D S H Y Q D V L K E V K A A A
 GAC AGC CAT TAC CAG GAC GTA CTC AAG GAG GTT AAA GCA GCG GCG

1260 1270
 S K V K A N L L S V E E A C S
 TCA AAA GTG AAG GCT AAC TTG CTA TCC GTA GAG GAA GCT TGC AGC

1280
 L T P P H S A K S K F G Y G A
 CTG ACG CCC CCA CAC TCA GCC AAA TCC AAG TTT GGT TAT GGG GCA

1290 1300
 K D V R C H A R K A V T H I N
 AAA GAC GTC CGT TGC CAT GCC AGA AAG GCC GTA ACC CAC ATC AAC

1310
 S V W K D L L E D N V T P I D
 TCC GTG TGG AAA GAC CTT CTG GAA GAC AAT GTA ACA CCA ATA GAC

1320 1330
 T T I M A K N E V F C V Q P E
 ACT ACC ATC ATG GCT AAG AAC GAG GTT TTC TGC GTT CAG CCT GAG

1340
 K G G R K P A R L I V F P D L
 AAG GGG GGT CGT AAG CCA GCT CGT CTC ATC GTG TTC CCC GAT CTG

1350 1360
 G V R V C E K M A L Y D V V T
 GGC GTG CGC GTG TGC GAA AAG ATG GCT TTG TAC GAC GTG GTT ACA

FIG. 5G

14/21

1370

K L P L A V M G S S Y G F Q Y
AAG CTC CCC TTG GCC GTG ATG GGA AGC TCC TAC GGA TTC CAA TAC

1380

S P G Q R V E F L V Q A W K S
TCA CCA GGA CAG CGG GTT GAA TTC CTC GTG CAA GCG TGG AAG TCC

1400

K K T P M G F S Y D T R C F D
AAG AAA ACC CCA ATG GGG TTC TCG TAT GAT ACC CGC TGC TTT GAC

1410

S T V T E S D I R T E E A I Y
TCC ACA GTC ACT GAG AGC GAC ATC CGT ACG GAG GAG GCA ATC TAC

1420

Q C C D L D P Q A R V A I K S
CAA TGT TGT GAC CTC GAC CCC CAA GCC CGC GTG GCC ATC AAG TCC

1430

L T E R L Y V G G P L T N S R
CTC ACC GAG AGG CTT TAT GTT GGG GGC CCT CTT ACC AAT TCA AGG

1440

G E N C G Y R R C R A S G V L
GGG GAG AAC TGC GGC TAT CGC AGG TGC CGC GCG AGC GGC GTA CTG

1450

T T S C G N T L T C Y I K A R
ACA ACT AGC TGT GGT AAC ACC CTC ACT TGC TAC ATC AAG GCC CGG

1460

A A C R A A G L Q D C T M L V
GCA GCC TGT CGA GCC GCA GGG CTC CAG GAC TGC ACC ATG CTC GTG

1470

C G D D L V V I C E S A G V Q
TGT GGC GAC GAC TTA GTC GTT ATC TGT GAA AGC GCG GGG GTC CAG

1480

E D A A S L R A F T E A M T R
GAG GAC GCG GCG AGC CTG AGA GCC TTC ACG GAG GCT ATG ACC AGG

1490

Y S A P P G D P P Q P E Y D L
TAC TCC GCC CCC CCT GGG GAC CCC CCA CAA CCA GAA TAC GAC TTG

1500

E L I T S C S S N V S V A H D
GAG CTC ATA ACA TCA TGC TCC AAC GTG TCA GTC GCC CAC GAC

1510

1520

1530

1540

1550

FIG. 5H

15/21

1560
 G A G K R V Y Y L T R D P T T
 GGC GCT GGA AAG AGG GTC TAC TAC CTC ACC CGT GAC CCT ACA ACC
 1580
 P L A R A A W E T A R H T P V
 CCC CTC GCG AGA GCT GCG TGG GAG ACA GCA AGA CAC ACT CCA GTC
 1590
 N S W L G N I I M F A P T L W
 AAT TCC TGG CTA GGC AAC ATA ATC ATG TTT GCC CCC ACA CTG TGG
 1610
 A R M I L M T H F F S V L I A
 GCG AGG ATG ATA CTG ATG ACC CAT TTC TTT AGC GTC CTT ATA GCC
 1620
 R D Q L E Q A L D C E I Y G A
 AGG GAC CAG CTT GAA CAG GCC CTC GAT TGC GAG ATC TAC GGG GCC
 1640
 C Y S I E P L D L P P I I Q R
 TGC TAC TCC ATA GAA CCA CTG GAT CTA CCT CCA ATC ATT CAA AGA
 1650
 L H G L S A F S L H S Y S P G
 CTC CAT GGC CTC AGC GCA TTT TCA CTC CAC AGT TAC TCT CCA GGT
 1670
 E I N R V A A C L R K L G V P
 GAA ATC AAT AGG GTG GCC GCA TGC CTC AGA AAA CTT GGG GTA CCG
 1680
 P L R A W R H R A R S V R A R
 CCC TTG CGA GCT TGG AGA CAC CGG GCC CGG AGC GTC CGC GCT AGG
 1700
 L L A R G G R A A I C G K Y L
 CTT CTG GCC AGA GGA GGC AGG GCT GCC ATA TGT GGC AAG TAC CTC
 1710
 F N W A V R T K L K L T P I A
 TTC AAC TGG GCA GTA AGA ACA AAG CTC AAA CTC ACT CCA ATA GCG
 1730
 A A G Q L D L S G W F T A G Y
 GCC GCT GGC CAG CTG GAC TTG TCC GGC TGG TTC ACG GCT GGC TAC
 1740
 S G G D I Y H S V S H A R P R
 AGC GGG GGA GAC ATT TAT CAC AGC GTG TCT CAT GCC CGG CCC CGC

FIG. 5T

16/21

1760
 W I W F C L L L L A A G V G I
 TGG ATC TGG TTT TGC CTA CTC CTG CTT GCT GCA GGG GTA GGC ATC

1770 1780
 Y L L P N R M S T N P K P Q R
 TAC CTC CTC CCC AAC CGA ATG AGC ACG AAT CCT AAA CCT CAA AGA

1790
 K T K R N T N R R P Q D V K F
 AAG ACC AAA CGT AAC ACC AAC CGG CGG CCG CAG GAC GTC AAG TTC

1800 1810
 P G G G Q I V G G V Y L L P R
 CCG GGT GGC GGT CAG ATC GTT GGT GGA GTT TAC TTG TTG CCG CGC

1820
 R G P R L G V R A T R K T S E
 AGG GGC CCT AGA TTG GGT GTG CGC GCG ACG AGA AAG ACT TCC GAG

1830 1840
 R S Q P R G R R Q P I P K A R
 CGG TCG CAA CCT CGA GGT AGA CGT CAG CCT ATC CCC AAG GCT CGT

1850
 R P E G R T W A Q P G Y P W P
 CGG CCC GAG GGC AGG ACC TGG GCT CAG CCC GGG TAC CCT TGG CCC

1860 1870
 L Y G N E G C G W A G W L L S
 CTC TAT GGC AAT GAG GGC TGC GGG TGG GCG GGA TGG CTC CTG TCT

1880
 P R G S R P S W G P T D P R R
 CCC CGT GGC TCT CGG CCT AGC TGG GGC CCC ACA GAC CCC CGG CGT

1890 1892
 R S R N L G K
 AGG TCG CGC AAT TTG GGT AAG

FIG. 5J

17/21

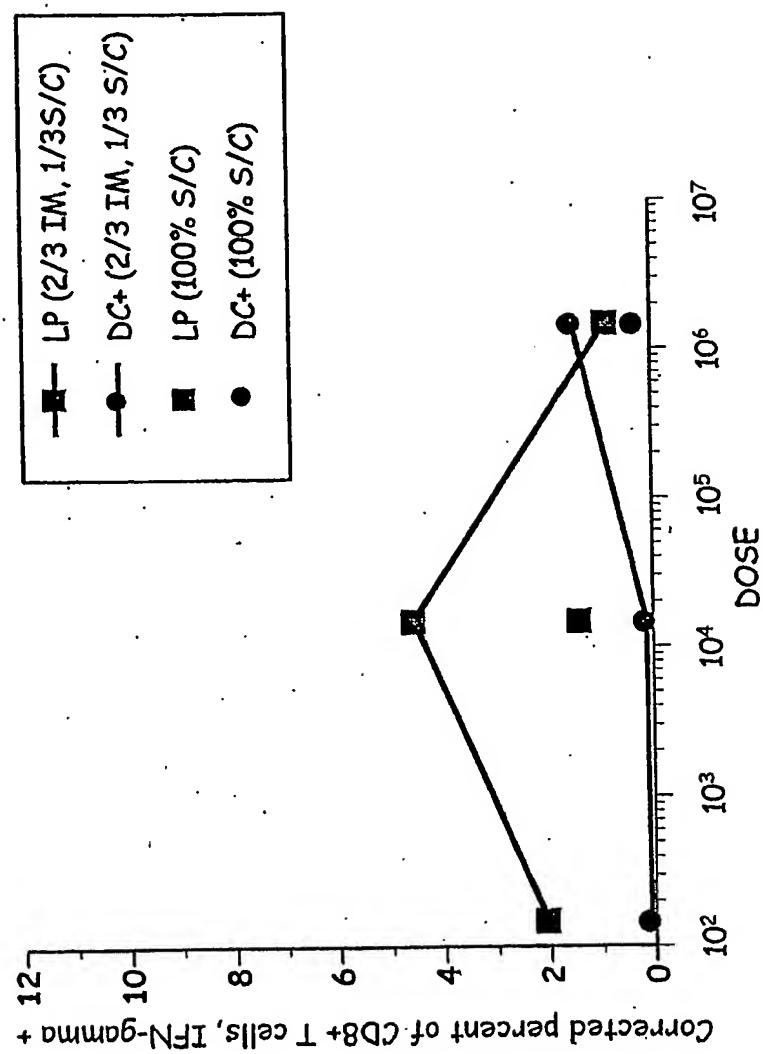


FIG. 6

18/21

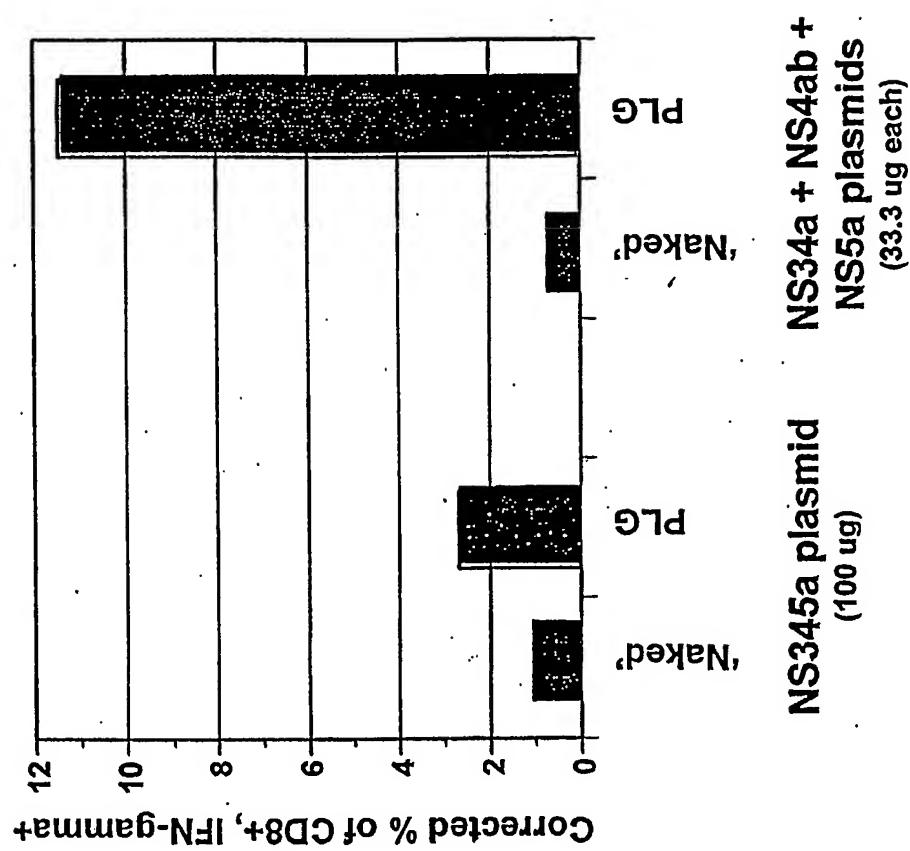


FIG. 7

19/21

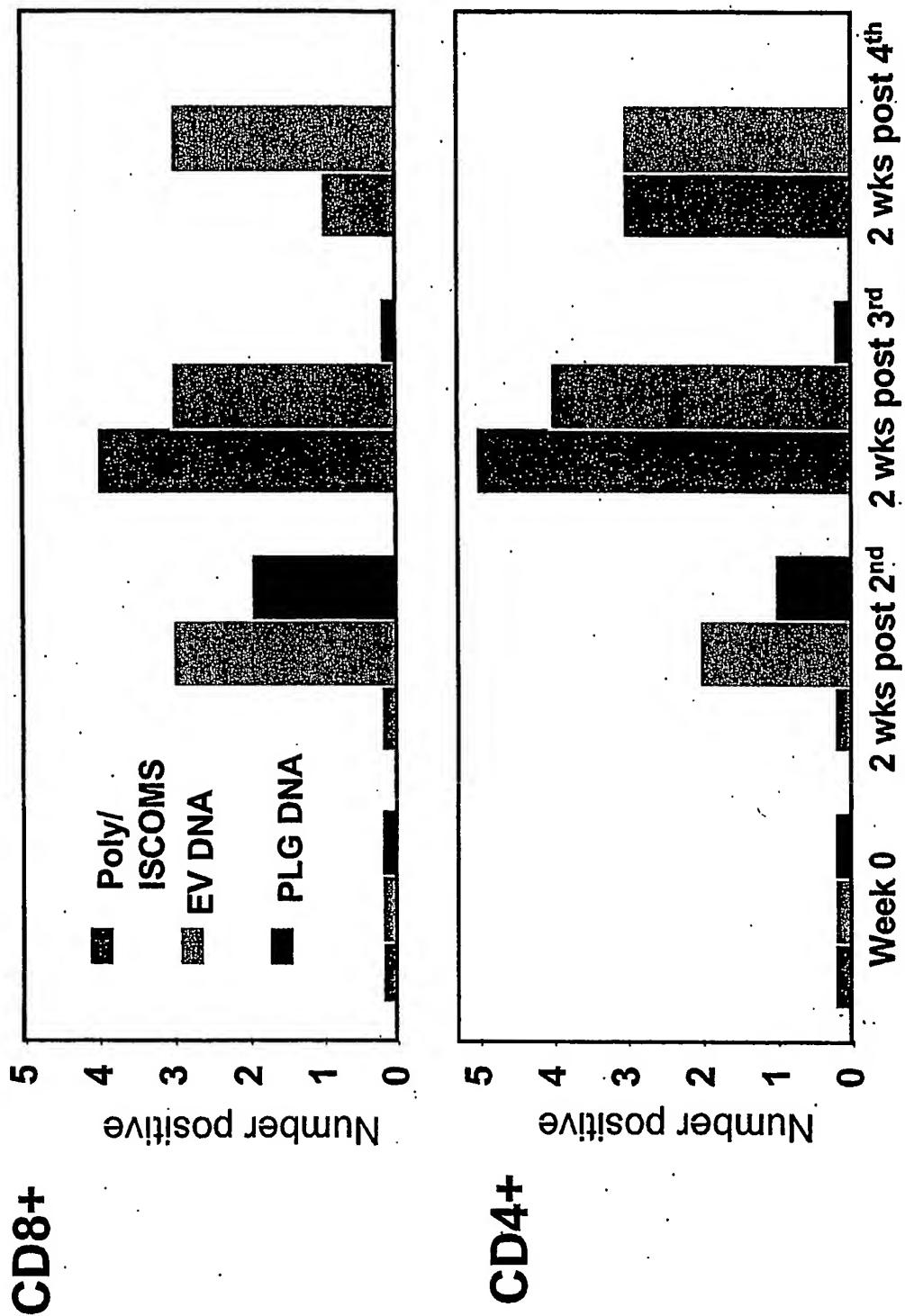


FIG. 8

20/21

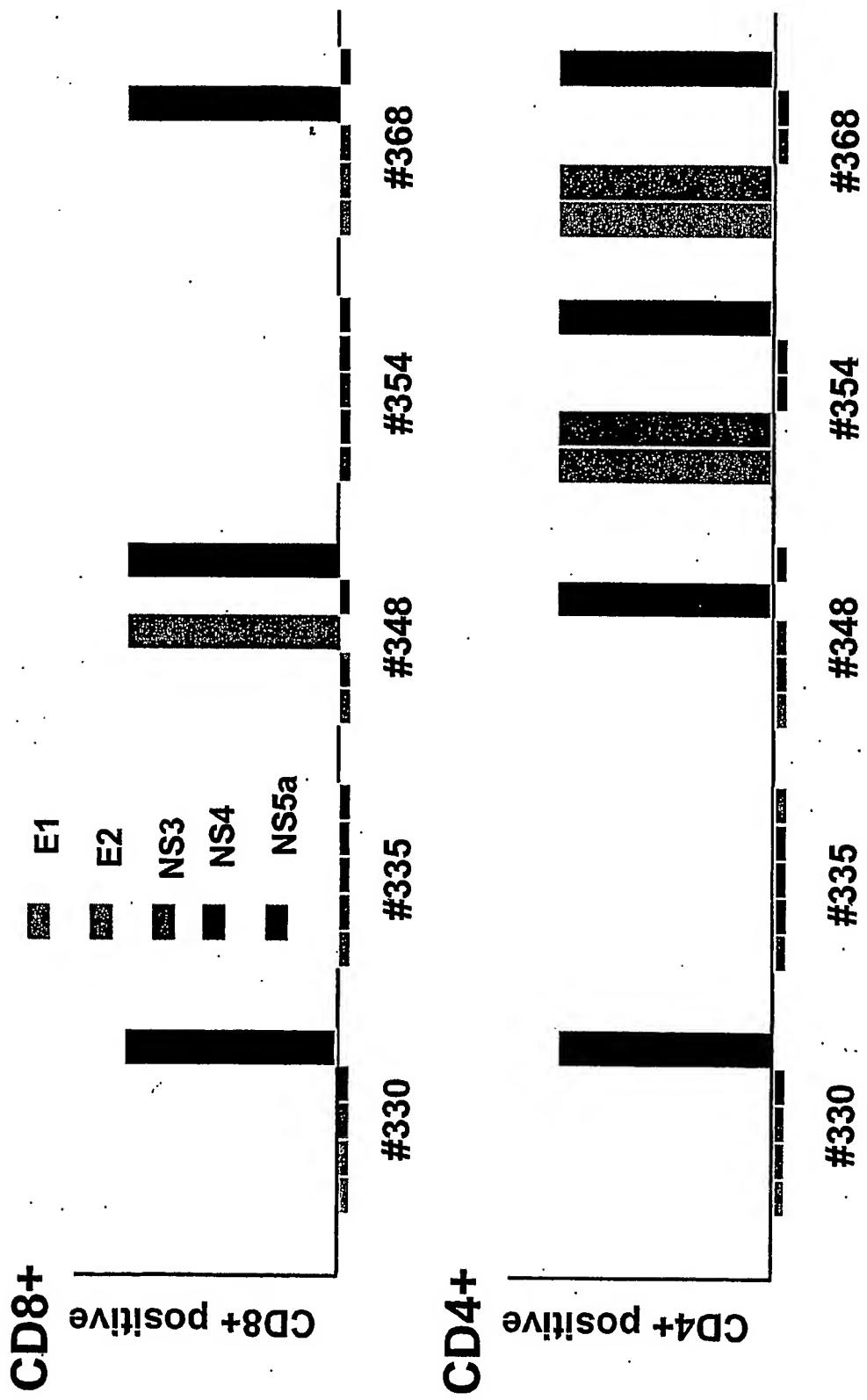


FIG. 9

21/21

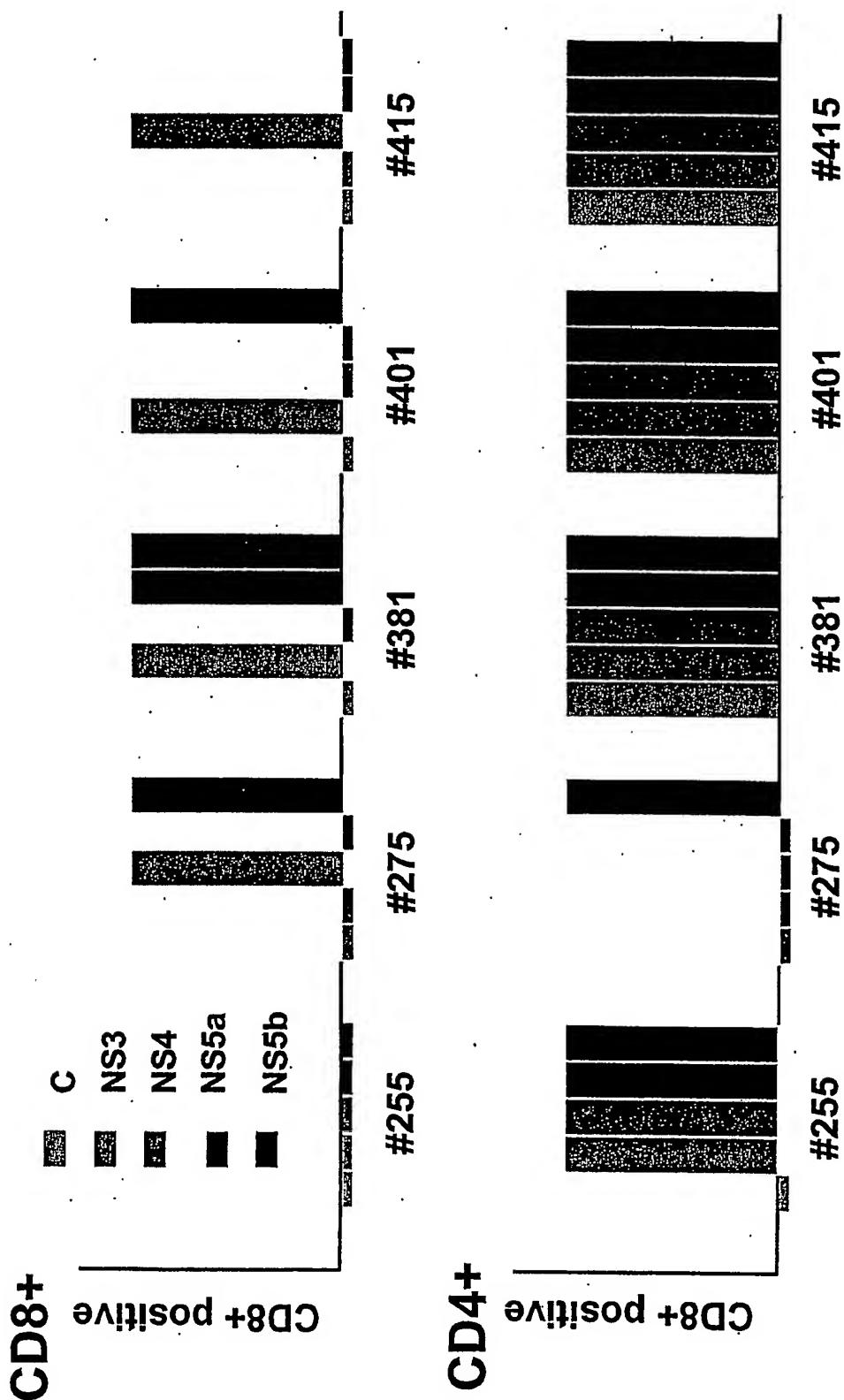


FIG. 10

SEQUENCE LISTING

<110> HOUGHTON, Michael
COATES, Steve
SELBY, Mark
PALIARD, Xavier

<120> ACTIVATION OF HCV-SPECIFIC T-CELLS

<130> 2300-1612.60,

<150> 10/281,341
<151> 2002-10-25

<160> 10

<170> PatentIn Ver. 2.0

<210> 1
<211> 9
<212> PRT
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: fusion protein
epitope

<400> 1

His Glu Tyr Pro Val Gly Ser Gln Leu
1 5

<210> 2

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: fusion protein
epitope

<400> 2

Ala Glu Leu Ile Glu Ala Asn Leu Leu Trp Arg Gln Glu Met Gly
1 5 10 15

<210> 3

<211> 1914

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: HCV-1 E1/E2/p7 region

<220>

<221> CDS

<222> (1)..(1911)

<400> 3

tct ttc tct atc ttc ctt ctg gcc ctg ctc tct tgc ttg act gtg ccc 48
Ser Phe Ser Ile Phe Leu Leu Ala Leu Leu Ser Cys Leu Thr Val Pro

1	5	10	15	
gct tcg gcc tac caa gtg cgc aac tcc acg ggg ctc tac cac gtc acc				96
Ala Ser Ala Tyr Gln Val Arg Asn Ser Thr Gly Leu Tyr His Val Thr				
20	25	30		
aat gat tgc cct aac tcg agt att gtg tac gag gcg gcc gat gcc atc				144
Asn Asp Cys Pro Asn Ser Ser Ile Val Tyr Glu Ala Ala Asp Ala Ile				
35	40	45		
ctg cac act ccg ggg tgc gtc cct tgc gtt cgc gag ggc aac gcc tcg				192
Leu His Thr Pro Gly Cys Val Pro Cys Val Arg Glu Gly Asn Ala Ser				
50	55	60		
agg tgt tgg gtg gcg atg acc cct acg gtg gcc acc agg gat ggc aaa				240
Arg Cys Trp Val Ala Met Thr Pro Thr Val Ala Thr Arg Asp Gly Lys				
65	70	75	80	
ctc ccc gcg acg cag ctt cga cgt cac atc gat ctg ctt gtc ggg agc				288
Leu Pro Ala Thr Gln Leu Arg Arg His Ile Asp Leu Leu Val Gly Ser				
85	90	95		
gcc acc ctc tgt tcg gcc ctc tac gtg ggg gac ctg tgc ggg tct gtc				336
Ala Thr Leu Cys Ser Ala Leu Tyr Val Gly Asp Leu Cys Gly Ser Val				
100	105	110		
ttt ctt gtc ggc caa ctg ttt acc ttc tct ccc agg cgc cac tgg acg				384
Phe Leu Val Gly Gln Leu Phe Thr Phe Ser Pro Arg Arg His Trp Thr				
115	120	125		
acg caa ggt tgc aat tgc tct atc tat ccc ggc cat ata acg ggt cac				432
Thr Gln Gly Cys Asn Cys Ser Ile Tyr Pro Gly His Ile Thr Gly His				
130	135	140		
cgc atg gca tgg gat atg atg aac tgg tcc cct acg acg gcg ttg				480
Arg Met Ala Trp Asp Met Met Asn Trp Ser Pro Thr Thr Ala Leu				
145	150	155	160	
gta atg gct cag ctg ctc cgg atc cca caa gcc atc ttg gac atg atc				528
Val Met Ala Gln Leu Leu Arg Ile Pro Gln Ala Ile Leu Asp Met Ile				
165	170	175		
gct ggt gct cac tgg gga gtc ctg gcg ata gcg tat ttc tcc atg				576
Ala Gly Ala His Trp Gly Val Leu Ala Gly Ile Ala Tyr Phe Ser Met				
180	185	190		
gtg ggg aac tgg gcg aag gtc ctg gta gtg ctg cta ttt gcc ggc				624
Val Gly Asn Trp Ala Lys Val Leu Val Leu Leu Phe Ala Gly				
195	200	205		
gtc gac gcg gaa acc cac gtc acc ggg gga agt gcc ggc cac act gtg				672
Val Asp Ala Glu Thr His Val Thr Gly Gly Ser Ala Gly His Thr Val				
210	215	220		
tct gga ttt gtt agc ctc ctc gca cca ggc gcc aag cag aac gtc cag				720
Ser Gly Phe Val Ser Leu Leu Ala Pro Gly Ala Lys Gln Asn Val Gln				
225	230	235	240	
ctg atc aac acc aac ggc agt tgg cac ctc aat agc acg gcc ctg aac				768
Leu Ile Asn Thr Asn Gly Ser Trp His Leu Asn Ser Thr Ala Leu Asn				
245	250	255		

tgc aat gat agc ctc aac acc ggc tgg ttg gca ggg ctt ttc tat cac Cys Asn Asp Ser Leu Asn Thr Gly Trp Leu Ala Gly Leu Phe Tyr His 260 265 270	816
cac aag ttc aac tct tca ggc tgt cct gag agg cta gcc agc tgc cga His Lys Phe Asn Ser Ser Gly Cys Pro Glu Arg Leu Ala Ser Cys Arg 275 280 285	864
ccc ctt acc gat ttt gac cag ggc tgg ggc cct atc agt tat gcc aac Pro Leu Thr Asp Phe Asp Gln Gly Trp Gly Pro Ile Ser Tyr Ala Asn 290 295 300	912
gga agc ggc ccc gac cag cgc ccc tac tgc tgg cac tac ccc cca aaa Gly Ser Gly Pro Asp Gln Arg Pro Tyr Cys Trp His Tyr Pro Pro Lys 305 310 315 320	960
cct tgc ggt att gtg ccc gcg aag agt gtg tgt ggt ccg gta tat tgc Pro Cys Gly Ile Val Pro Ala Lys Ser Val Cys Gly Pro Val Tyr Cys 325 330 335	1008
ttc act ccc agc ccc gtg gtg gga acg acc gac agg tcg ggc gcg Phe Thr Pro Ser Pro Val Val Val Gly Thr Thr Asp Arg Ser Gly Ala 340 345 350	1056
ccc acc tac agc tgg ggt gaa aat gat acg gac gtc ttc gtc ctt aac Pro Thr Tyr Ser Trp Gly Glu Asn Asp Thr Asp Val Phe Val Leu Asn 355 360 365	1104
aat acc agg cca ccg ctg ggc aat tgg ttc ggt tgt acc tgg atg aac Asn Thr Arg Pro Pro Leu Gly Asn Trp Phe Gly Cys Thr Trp Met Asn 370 375 380	1152
tca act gga ttc acc aaa gtg tgc gga gcg cct cct tgt gtc atc gga Ser Thr Gly Phe Thr Lys Val Cys Gly Ala Pro Pro Cys Val Ile Gly 385 390 395 400	1200
ggg gcg ggc aac aac acc ctg cac tgc ccc act gat tgc ttc cgc aag Gly Ala Gly Asn Asn Thr Leu His Cys Pro Thr Asp Cys Phe Arg Lys 405 410 415	1248
cat ccg gac gcc aca tac tct cgg tgc ggc tcc ggt ccc tgg atc aca His Pro Asp Ala Thr Tyr Ser Arg Cys Gly Ser Gly Pro Trp Ile Thr 420 425 430	1296
ccc agg tgc ctg gtc gac tac ccg tat agg ctt tgg cat tat cct tgt Pro Arg Cys Leu Val Asp Tyr Pro Tyr Arg Leu Trp His Tyr Pro Cys 435 440 445	1344
acc atc aac tac act ata ttt aaa atc agg atg tac gtg gga ggg gtc Thr Ile Asn Tyr Thr Ile Phe Lys Ile Arg Met Tyr Val Gly Gly Val 450 455 460	1392
gag cac agg ctg gaa gct gcc tgc aac tgg acg ccg ggc gaa cgt tgc Glu His Arg Leu Glu Ala Ala Cys Asn Trp Thr Arg Gly Glu Arg Cys 465 470 475 480	1440
gat ctg gaa gat agg gac agg tcc gag ctc agc ccg tta ctg ctg acc Asp Leu Glu Asp Arg Asp Arg Ser Glu Leu Ser Pro Leu Leu Thr 485 490 495	1488
act aca cag tgg cag gtc ctc ccg tgt tcc ttc aca acc ctg cca gcc	1536

Thr Thr Gln Trp Gln Val Leu Pro Cys Ser Phe Thr Thr Leu Pro Ala			
500	505	510	
ttg tcc acc ggc ctc atc cac ctc cac cag aac att gtg gac gtg cag	1584		
Leu Ser Thr Gly Leu Ile His Leu His Gln Asn Ile Val Asp Val Gln			
515	520	525	
tac ttg tac ggg gtg ggg tca agc atc gcg tcc tgg gcc att aag tgg	1632		
Tyr Leu Tyr Gly Val Gly Ser Ser Ile Ala Ser Trp Ala Ile Lys Trp			
530	535	540	
gag tac gtc gtc ctc ctg ttc ctt ctg ctt gca gac gcg cgc gtc tgc	1680		
Glu Tyr Val Val Leu Leu Phe Leu Leu Ala Asp Ala Arg Val Cys			
545	550	555	560
tcc tgc ttg tgg atg atg cta ctc ata tcc caa gcg gaa gcg gct ttg	1728		
Ser Cys Leu Trp Met Met Leu Leu Ile Ser Gln Ala Glu Ala Ala Leu			
565	570	575	
gag aac ctc gta ata ctt aat gca gca tcc ctg gcc ggg acg cac ggt	1776		
Glu Asn Leu Val Ile Leu Asn Ala Ala Ser Leu Ala Gly Thr His Gly			
580	585	590	
ctt gta tcc ttc ctc gtg ttc tgc ttt gca tgg tat ctg aag ggt	1824		
Leu Val Ser Phe Leu Val Phe Phe Cys Phe Ala Trp Tyr Leu Lys Gly			
595	600	605	
aag tgg gtg ccc gga gcg gtc tac acc ttc tac ggg atg tgg cct ctc	1872		
Lys Trp Val Pro Gly Ala Val Tyr Thr Phe Tyr Gly Met Trp Pro Leu			
610	615	620	
ctc ctg ctc ctg ttg gcg ttg ccc cag cgg gcg tac gcg taa	1914		
Leu Leu Leu Leu Ala Leu Pro Gln Arg Ala Tyr Ala			
625	630	635	
<210> 4			
<211> 637			
<212> PRT			
<213> Artificial Sequence			
<220>			
<223> Description of Artificial Sequence: HCV-1 E1/E2/p7 region			
<400> 4			
Ser Phe Ser Ile Phe Leu Leu Ala Leu Leu Ser Cys Leu Thr Val Pro			
1 5 10 15			
Ala Ser Ala Tyr Gin Val Arg Asn Ser Thr Gly Leu Tyr His Val Thr			
20 25 30			
Asn Asp Cys Pro Asn Ser Ser Ile Val Tyr Glu Ala Ala Asp Ala Ile			
35 40 45			
Leu His Thr Pro Gly Cys Val Pro Cys Val Arg Glu Gly Asn Ala Ser			
50 55 60			
Arg Cys Trp Val Ala Met Thr Pro Thr Val Ala Thr Arg Asp Gly Lys			
65 70 75 80			
Leu Pro Ala Thr Gln Leu Arg Arg His Ile Asp Leu Leu Val Gly Ser			

85	90	95
Ala Thr Leu Cys Ser Ala Leu Tyr Val Gly Asp Leu Cys Gly Ser Val		
100	105	110
Phe Leu Val Gly Gln Leu Phe Thr Phe Ser Pro Arg Arg His Trp Thr		
115	120	125
Thr Gln Gly Cys Asn Cys Ser Ile Tyr Pro Gly His Ile Thr Gly His		
130	135	140
Arg Met Ala Trp Asp Met Met Asn Trp Ser Pro Thr Thr Ala Leu		
145	150	155
Val Met Ala Gln Leu Leu Arg Ile Pro Gln Ala Ile Leu Asp Met Ile		
165	170	175
Ala Gly Ala His Trp Gly Val Leu Ala Gly Ile Ala Tyr Phe Ser Met		
180	185	190
Val Gly Asn Trp Ala Lys Val Leu Val Val Leu Leu Phe Ala Gly		
195	200	205
Val Asp Ala Glu Thr His Val Thr Gly Gly Ser Ala Gly His Thr Val		
210	215	220
Ser Gly Phe Val Ser Leu Leu Ala Pro Gly Ala Lys Gln Asn Val Gln		
225	230	235
Leu Ile Asn Thr Asn Gly Ser Trp His Leu Asn Ser Thr Ala Leu Asn		
245	250	255
Cys Asn Asp Ser Leu Asn Thr Gly Trp Leu Ala Gly Leu Phe Tyr His		
260	265	270
His Lys Phe Asn Ser Ser Gly Cys Pro Glu Arg Leu Ala Ser Cys Arg		
275	280	285
Pro Leu Thr Asp Phe Asp Gln Gly Trp Gly Pro Ile Ser Tyr Ala Asn		
290	295	300
Gly Ser Gly Pro Asp Gln Arg Pro Tyr Cys Trp His Tyr Pro Pro Lys		
305	310	315
Pro Cys Gly Ile Val Pro Ala Lys Ser Val Cys Gly Pro Val Tyr Cys		
325	330	335
Phe Thr Pro Ser Pro Val Val Val Gly Thr Thr Asp Arg Ser Gly Ala		
340	345	350
Pro Thr Tyr Ser Trp Gly Glu Asn Asp Thr Asp Val Phe Val Leu Asn		
355	360	365
Asn Thr Arg Pro Pro Leu Gly Asn Trp Phe Gly Cys Thr Trp Met Asn		
370	375	380
Ser Thr Gly Phe Thr Lys Val Cys Gly Ala Pro Pro Cys Val Ile Gly		
385	390	395
Gly Ala Gly Asn Asn Thr Leu His Cys Pro Thr Asp Cys Phe Arg Lys		
405	410	415

His Pro Asp Ala Thr Tyr Ser Arg Cys Gly Ser Gly Pro Trp Ile Thr
 420 425 430

Pro Arg Cys Leu Val Asp Tyr Pro Tyr Arg Leu Trp His Tyr Pro Cys
 435 440 445

Thr Ile Asn Tyr Thr Ile Phe Lys Ile Arg Met Tyr Val Gly Gly Val
 450 455 460

Glu His Arg Leu Glu Ala Ala Cys Asn Trp Thr Arg Gly Glu Arg Cys
 465 470 475 480

Asp Leu Glu Asp Arg Asp Arg Ser Glu Leu Ser Pro Leu Leu Leu Thr
 485 490 495

Thr Thr Gln Trp Gln Val Leu Pro Cys Ser Phe Thr Thr Leu Pro Ala
 500 505 510

Leu Ser Thr Gly Leu Ile His Leu His Gln Asn Ile Val Asp Val Gln
 515 520 525

Tyr Leu Tyr Gly Val Gly Ser Ser Ile Ala Ser Trp Ala Ile Lys Trp
 530 535 540

Glu Tyr Val Val Leu Leu Phe Leu Leu Ala Asp Ala Arg Val Cys
 545 550 555 560

Ser Cys Leu Trp Met Met Leu Leu Ile Ser Gln Ala Glu Ala Ala Leu
 565 570 575

Glu Asn Leu Val Ile Leu Asn Ala Ala Ser Leu Ala Gly Thr His Gly
 580 585 590

Leu Val Ser Phe Leu Val Phe Phe Cys Phe Ala Trp Tyr Leu Lys Gly
 595 600 605

Lys Trp Val Pro Gly Ala Val Tyr Thr Phe Tyr Gly Met Trp Pro Leu
 610 615 620

Leu Leu Leu Leu Ala Leu Pro Gln Arg Ala Tyr Ala
 625 630 635

<210> 5

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: consensus sequence

<400> 5

Gly Ser Ala Ala Arg Thr Thr Ser Gly Phe Val Ser Leu Phe Ala Pro
 1 5 10 15

Gly Ala Lys Gln Asn
 20

<210> 6

<211> 20

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: exemplary CpG oligonucleotide

<400> 6
tccatgacgt tcctgacgtt

20

```
<210> 7
<211> 5676
<212> DNA
<213> Artificial Sequence
```

<220>
<223> Description of Artificial Sequence: representative
NS345Core fusion protein

<220>
<221> CDS
<222> (1)..(5676)

<400> 7
atg gct gca tat gca gct cag ggc tat aag gtg cta gta ctc aac ccc 48
Met Ala Ala Tyr Ala Ala Gln Gly Tyr Lys Val Leu Val Leu Asn Pro
1 5 10 15

tct gtt gct gca aca ctg ggc ttt ggt gct tac atg tcc aag gct cat	96
Ser Val Ala Ala Thr Leu Gly Phe Gly Ala Tyr Met Ser Lys Ala His	
20 25 30	

ggg atc gat cct aac atc agg acc ggg gtg aga aca att acc act ggc 144
Gly Ile Asp Pro Asn Ile Arg Thr Gly Val Arg Thr Ile Thr Thr Gly
35 40 45

```

  agc ccc atc acg tac tcc acc tac ggc aag ttc ctt gcc gac ggc ggg  192
  Ser Pro Ile Thr Tyr Ser Thr Tyr Gly Lys Phe Leu Ala Asp Gly Gly
      50          55          60

```

```

tgc tcg ggg ggc gct tat gac ata ata att tgt gac gag tgc cac tcc 240
Cys Ser Gly Gly Ala Tyr Asp Ile Ile Ile Cys Asp Glu Cys His Ser
   65           70           75           80

```

acg gat gcc aca tcc atc ttg ggc att ggc act gtc ctt gac caa gca 288
 Thr Asp Ala Thr Ser Ile Leu Gly Ile Gly Thr Val Leu Asp Gln Ala
 85. 90 95

gag act gcg ggg gcg aga ctg gtt gtg ctc gcc acc gcc acc cct ccg 336
 Glu Thr Ala Gly Ala Arg Leu Val Val Leu Ala Thr Ala Thr Pro Pro
 100 105 110

ggc tcc gtc act gtg ccc cat ccc aac atc gag gag gtt gct ctg tcc 384
Gly Ser Val Thr Val Pro His Pro Asn Ile Glu Glu Val Ala Leu Ser
115 120 125

acc acc gga gag atc cct ttt tac ggc aag gct atc ccc ctc gaa gta 432
 Thr Thr Gly Glu Ile Pro Phe Tyr Gly Lys Ala Ile Pro Leu Glu Val
 130 135 140

atc aag ggg ggg aga cat ctc atc ttc tgt cat tca aag aag aag tgc 480

Ile Lys Gly Gly Arg His Leu Ile Phe Cys His Ser Lys Lys Lys Cys				
145	150	155	160	
gac gaa ctc gcc gca aag ctg gtc gca ttg ggc atc aat gcc gtg gcc				528
Asp Glu Leu Ala Ala Lys Leu Val Ala Leu Gly Ile Asn Ala Val Ala				
165	170	175		
tac tac cgc ggt ctt gac gtg tcc gtc atc ccg acc agc ggc gat gtt				576
Tyr Tyr Arg Gly Leu Asp Val Ser Val Ile Pro Thr Ser Gly Asp Val				
180	185	190		
gtc gtc gtg gca acc gat gcc ctc atg acc ggc tat acc ggc gac ttc				624
Val Val Val Ala Thr Asp Ala Leu Met Thr Gly Tyr Thr Gly Asp Phe				
195	200	205		
gac tcg gtg ata gac tgc aat acg tgt gtc acc cag aca gtc gat ttc				672
Asp Ser Val Ile Asp Cys Asn Thr Cys Val Thr Gln Thr Val Asp Phe				
210	215	220		
agc ctt gac cct acc ttc acc att gag aca atc acg ctc ccc caa gat				720
Ser Leu Asp Pro Thr Phe Thr Ile Glu Thr Ile Thr Leu Pro Gln Asp				
225	230	235	240	
gct gtc tcc cgc act caa cgt cgg ggc agg act ggc agg ggg aag cca				768
Ala Val Ser Arg Thr Gln Arg Arg Gly Arg Thr Gly Arg Gly Lys Pro				
245	250	255		
ggc atc tac aga ttt gtg gca ccg ggg gag cgc ccc tcc ggc atg ttc				816
Gly Ile Tyr Arg Phe Val Ala Pro Gly Glu Arg Pro Ser Gly Met Phe				
260	265	270		
gac tcg tcc gtc ctc tgt gag tgc tat gac gca ggc tgt gct tgg tat				864
Asp Ser Ser Val Leu Cys Glu Cys Tyr Asp Ala Gly Cys Ala Trp Tyr				
275	280	285		
gag ctc acg ccc gcc gag act aca gtt agg cta cga gcg tac atg aac				912
Glu Leu Thr Pro Ala Glu Thr Val Arg Leu Arg Ala Tyr Met Asn				
290	295	300		
acc ccg ggg ctt ccc gtg tgc cag gac cat ctt gaa ttt tgg gag ggc				960
Thr Pro Gly Leu Pro Val Cys Gln Asp His Leu Glu Phe Trp Glu Gly				
305	310	315	320	
gtc ttt aca ggc ctc act cat ata gat gcc cac ttt cta tcc cag aca				1008
Val Phe Thr Gly Leu Thr His Ile Asp Ala His Phe Leu Ser Gln Thr				
325	330	335		
aag cag agt ggg gag aac ctt cct tac ctg gta gcg tac caa gcc acc				1056
Lys Gln Ser Gly Glu Asn Leu Pro Tyr Leu Val Ala Tyr Gln Ala Thr				
340	345	350		
gtg tgc gct agg gct caa gcc cct ccc cca tcg tgg gac cag atg tgg				1104
Val Cys Ala Arg Ala Gln Ala Pro Pro Ser Trp Asp Gln Met Trp				
355	360	365		
aag tgt ttg att cgc ctc aag ccc acc ctc cat ggg cca aca ccc ctg				1152
Lys Cys Leu Ile Arg Leu Lys Pro Thr Leu His Gly Pro Thr Pro Leu				
370	375	380		
cta tac aga ctg ggc gct gtt cag aat gaa atc acc ctg acg cac cca				1200
Leu Tyr Arg Leu Gly Ala Val Gln Asn Glu Ile Thr Leu Thr His Pro				

385	390	395	400	
gtc acc aaa tac atc atg aca tgc atg tcg gcc gac ctg gag gtc gtc				1248
Val Thr Lys Tyr Ile Met Thr Cys Met Ser Ala Asp Leu Glu Val Val				
405	410	415		
acg agc acc tgg gtg ctc gtt ggc ggc gtc ctg gct gct ttg gcc gcg				1296
Thr Ser Thr Trp Val Leu Val Gly Gly Val Leu Ala Ala Leu Ala Ala				
420	425	430		
tat tgc ctg tca aca ggc tgc gtg gtc ata gtg ggc agg gtc gtc ttg				1344
Tyr Cys Leu Ser Thr Gly Cys Val Val Ile Val Gly Arg Val Val Leu				
435	440	445		
tcc ggg aag ccg gca atc ata cct gac agg gaa gtc ctc tac cga gag				1392
Ser Gly Lys Pro Ala Ile Ile Pro Asp Arg Glu Val Leu Tyr Arg Glu				
450	455	460		
ttc gat gag atg gaa gag tgc tct cag cac tta ccg tac atc gag caa				1440
Phe Asp Glu Met Glu Glu Cys Ser Gln His Leu Pro Tyr Ile Glu Gln				
465	470	475	480	
ggg atg atg ctc gcc gag cag ttc aag cag aag gcc ctc ggc ctc ctg				1488
Gly Met Met Leu Ala Glu Gln Phe Lys Gln Lys Ala Leu Gly Leu Leu				
485	490	495		
cag acc gcg tcc cgt cag gca gag gtt atc gcc cct gct gtc cag acc				1536
Gln Thr Ala Ser Arg Gln Ala Glu Val Ile Ala Pro Ala Val Gln Thr				
500	505	510		
aac tgg caa aaa ctc gag acc ttc tgg gcg aag cat atg tgg aac ttc				1584
Asn Trp Gln Lys Leu Glu Thr Phe Trp Ala Lys His Met Trp Asn Phe				
515	520	525		
atc agt ggg ata caa tac ttg gcg ggc ttg tca acg ctg cct ggt aac				1632
Ile Ser Gly Ile Gln Tyr Leu Ala Gly Leu Ser Thr Leu Pro Gly Asn				
530	535	540		
ccc gcc att gct tca ttg atg gct ttt aca gct gct gtc acc agc cca				1680
Pro Ala Ile Ala Ser Leu Met Ala Phe Thr Ala Ala Val Thr Ser Pro				
545	550	555	560	
cta acc act agc caa acc ctc ctc ttc aac ata ttg ggg ggg tgg gtg				1728
Leu Thr Thr Ser Gln Thr Leu Leu Phe Asn Ile Leu Gly Gly Trp Val				
565	570	575		
gct gcc cag ctc gcc gcc ccc ggt gcc gct act gcc ttt gtg ggc gct				1776
Ala Ala Gln Leu Ala Ala Pro Gly Ala Ala Thr Ala Phe Val Gly Ala				
580	585	590		
ggc tta gct ggc gcc atc ggc agt gtt gga ctg ggg aag gtc ctc				1824
Gly Leu Ala Gly Ala Ala Ile Gly Ser Val Gly Leu Gly Lys Val Leu				
595	600	605		
ata gac atc ctt gca ggg tat ggc gcg ggc gtg gcg gga gct ctt gtg				1872
Ile Asp Ile Leu Ala Gly Tyr Gly Ala Gly Val Ala Gly Ala Leu Val				
610	615	620		
gca ttc aag atc atg agc ggt gag gtc ccc tcc acg gag gac ctg gtc				1920
Ala Phe Lys Ile Met Ser Gly Glu Val Pro Ser Thr Glu Asp Leu Val				
625	630	635	640	

aat cta ctg ccc gcc atc ctc tcg ccc gga gcc ctc gta gtc ggc gtg Asn Leu Leu Pro Ala Ile Leu Ser Pro Gly Ala Leu Val Val Gly Val 645 650 655	1968
gtc tgt gca gca ata ctg cgc cgg cac gtt ggc cgg gag ggg gca Val Cys Ala Ala Ile Leu Arg Arg His Val Gly Pro Gly Glu Gly Ala 660 665 670	2016
gtg cag tgg atg aac cgg ctg ata gcc ttc gcc tcc cgg ggg aac cat Val Gln Trp Met Asn Arg Leu Ile Ala Phe Ala Ser Arg Gly Asn His 675 680 685	2064
gtt tcc ccc acg cac tac gtg cgg gag agc gat gca gct gcc cgc gtc Val Ser Pro Thr His Tyr Val Pro Glu Ser Asp Ala Ala Arg Val 690 695 700	2112
act gcc ata ctc agc agc ctc act gta acc cag ctc ctg agg cga ctg Thr Ala Ile Leu Ser Ser Leu Thr Val Thr Gln Leu Leu Arg Arg Leu 705 710 715 720	2160
cac cag tgg ata agc tcg gag tgt acc act cca tgc tcc ggt tcc tgg His Gln Trp Ile Ser Ser Glu Cys Thr Thr Pro Cys Ser Gly Ser Trp 725 730 735	2208
cta agg gac atc tgg gac tgg ata tgc gag gtg ttg agc gac ttt aag Leu Arg Asp Ile Trp Asp Trp Ile Cys Glu Val Leu Ser Asp Phe Lys 740 745 750	2256
acc tgg cta aaa gct aag ctc atg cca cag ctg cct ggg atc ccc ttt Thr Trp Leu Lys Ala Lys Leu Met Pro Gln Leu Pro Gly Ile Pro Phe 755 760 765	2304
gtg tcc tgc cag cgc ggg tat aag ggg gtc tgg cga ggg gac ggc atc Val Ser Cys Gln Arg Gly Tyr Lys Gly Val Trp Arg Gly Asp Gly Ile 770 775 780	2352
atg cac act cgc tgc cac tgt gga gct gag atc act gga cat gtc aaa Met His Thr Arg Cys His Cys Gly Ala Glu Ile Thr Gly His Val Lys 785 790 795 800	2400
aac ggg acg atg agg atc gtc ggt cct agg acc tgc agg aac atg tgg Asn Gly Thr Met Arg Ile Val Gly Pro Arg Thr Cys Arg Asn Met Trp 805 810 815	2448
agt ggg acc ttc ccc att aat gcc tac acc acg ggc ccc tgt acc ccc Ser Gly Thr Phe Pro Ile Asn Ala Tyr Thr Thr Gly Pro Cys Thr Pro 820 825 830	2496
ctt cct gcg ccg aac tac acg ttc gcg cta tgg agg gtc tct gca gag Leu Pro Ala Pro Asn Tyr Thr Phe Ala Leu Trp Arg Val Ser Ala Glu 835 840 845	2544
gaa tac gtg gag ata agg cag gtg ggg gac ttc cac tac gtg acg ggt Glu Tyr Val Glu Ile Arg Gln Val Gly Asp Phe His Tyr Val Thr Gly 850 855 860	2592
atg act act gac aat ctt aaa tgc ccg tgc cag gtc cca tcg ccc gaa Met Thr Thr Asp Asn Leu Lys Cys Pro Cys Gln Val Pro Ser Pro Glu 865 870 875 880	2640

ttt ttc aca gaa ttg gac ggg gtg cgc cta cat agg ttt gcg ccc ccc Phe Phe Thr Glu Leu Asp Gly Val Arg Leu His Arg Phe Ala Pro Pro 885 890 895	2688
tgc aag ccc ttg ctg cgg gag gta tca ttc aga gta gga ctc cac Cys Lys Pro Leu Leu Arg Glu Glu Val Ser Phe Arg Val Gly Leu His 900 905 910	2736
gaa tac ccg gta ggg tcg caa tta cct tgc gag ccc gaa ccg gac gtg Glu Tyr Pro Val Gly Ser Gln Leu Pro Cys Glu Pro Asp Val 915 920 925	2784
gcc gtg ttg acg tcc atg ctc act gat ccc tcc cat ata aca gca gag Ala Val Leu Thr Ser Met Leu Thr Asp Pro Ser His Ile Thr Ala Glu 930 935 940	2832
gcg gcc ggg cga agg ttg gcg agg gga tca ccc ccc tct gtg gcc agc Ala Ala Gly Arg Arg Leu Ala Arg Gly Ser Pro Pro Ser Val Ala Ser 945 950 955 960	2880
tcc tcg gct agc cag cta tcc gct cca tct ctc aag gca act tgc acc Ser Ser Ala Ser Gln Leu Ser Ala Pro Ser Leu Lys Ala Thr Cys Thr 965 970 975	2928
gct aac cat gac tcc cct gat gct gag ctc ata gag gcc aac ctc cta Ala Asn His Asp Ser Pro Asp Ala Glu Leu Ile Glu Ala Asn Leu Leu 980 985 990	2976
tgg agg cag gag atg ggc ggc aac atc acc agg gtt gag tca gaa aac Trp Arg Gln Glu Met Gly Gly Asn Ile Thr Arg Val Glu Ser Glu Asn 995 1000 1005	3024
aaa gtg gtg att ctg gac tcc ttc gat ccg ctt gtg gcg gag gag gag Lys Val Val Ile Leu Asp Ser Phe Asp Pro Leu Val Ala Glu Glu Asp 1010 1015 1020	3072
gag cgg gag atc tcc gta ccc gca gaa atc ctg cgg aag tct cgg aga Glu Arg Glu Ile Ser Val Pro Ala Glu Ile Leu Arg Lys Ser Arg Arg 1025 1030 1035 1040	3120
ttc gcc cag gcc ctg ccc gtt tgg gcg cgg ccg gac tat aac ccc ccg Phe Ala Gln Ala Leu Pro Val Trp Ala Arg Pro Asp Tyr Asn Pro Pro 1045 1050 1055	3168
cta gtg gag acg tgg aaa aag ccc gac tac gaa cca cct gtg gtc cat Leu Val Glu Thr Trp Lys Lys Pro Asp Tyr Glu Pro Pro Val Val His 1060 1065 1070	3216
ggc tgc ccg ctt cca cct cca aag tcc cct cct gtg cct ccg cct cgg Gly Cys Pro Leu Pro Pro Lys Ser Pro Pro Val Pro Pro Pro Arg 1075 1080 1085	3264
aag aag cgg acg gtg gtc ctc act gaa tca acc cta tct act gcc ttg Lys Lys Arg Thr Val Val Leu Thr Glu Ser Thr Leu Ser Thr Ala Leu 1090 1095 1100	3312
gcc gag ctc gcc acc aga agc ttt ggc agc tcc tca act tcc ggc att Ala Glu Leu Ala Thr Arg Ser Phe Gly Ser Ser Thr Ser Gly Ile 1105 1110 1115 1120	3360
acg ggc gac aat acg aca aca tcc tct gag ccc gcc cct tct ggc tgc	3408

Thr Gly Asp Asn Thr Thr Thr Ser Ser Glu Pro Ala Pro Ser Gly Cys		
1125	1130	1135
ccc ccc gac tcc gac gct gag tcc tat tcc tcc atg ccc ccc ctg gag	3456	
Pro Pro Asp Ser Asp Ala Glu Ser Tyr Ser Ser Met Pro Pro Leu Glu		
1140	1145	1150
ggg gag cct ggg gat ccg gat ctt agc gac ggg tca tgg tca acg gtc	3504	
Gly Glu Pro Gly Asp Pro Asp Leu Ser Asp Gly Ser Trp Ser Thr Val		
1155	1160	1165
agt agt gag gcc aac gcg gag gat gtc gtg tgc tgc tca atg tct tac	3552	
Ser Ser Glu Ala Asn Ala Glu Asp Val Val Cys Cys Ser Met Ser Tyr		
1170	1175	1180
tct tgg aca ggc gca ctc gtc acc ccg tgc gcc gcg gaa gaa cag aaa	3600	
Ser Trp Thr Gly Ala Leu Val Thr Pro Cys Ala Ala Glu Glu Gln Lys		
1185	1190	1195
ctg ccc atc aat gca cta agc aac tcg ttg cta cgt cac cac aat ttg	3648	
Leu Pro Ile Asn Ala Leu Ser Asn Ser Leu Leu Arg His His Asn Leu		
1205	1210	1215
gtg tat tcc acc acc tca cgc agt gct tgc caa agg cag aag aaa gtc	3696	
Val Tyr Ser Thr Ser Arg Ser Ala Cys Gln Arg Gln Lys Lys Val		
1220	1225	1230
aca ttt gac aga ctg caa gtt ctg gac agc cat tac cag gac gta ctc	3744	
Thr Phe Asp Arg Leu Gln Val Leu Asp Ser His Tyr Gln Asp Val Leu		
1235	1240	1245
aag gag gtt aaa gca gcg gcg tca aaa gtg aag gct aac ttg cta tcc	3792	
Lys Glu Val Lys Ala Ala Ser Lys Val Lys Ala Asn Leu Leu Ser		
1250	1255	1260
gta gag gaa gct tgc agc ctg acg ccc cca cac tca gcc aaa tcc aag	3840	
Val Glu Glu Ala Cys Ser Leu Thr Pro Pro His Ser Ala Lys Ser Lys		
1265	1270	1275
ttt ggt tat ggg gca aaa gac gtc cgt tgc cat gcc aga aag gcc gta	3888	
Phe Gly Tyr Gly Ala Lys Asp Val Arg Cys His Ala Arg Lys Ala Val		
1285	1290	1295
acc cac atc aac tcc gtg tgg aaa gac ctt ctg gaa gac aat gta aca	3936	
Thr His Ile Asn Ser Val Trp Lys Asp Leu Leu Glu Asp Asn Val Thr		
1300	1305	1310
cca ata gac act acc atc atg gct aag aac gag gtt ttc tgc gtt cag	3984	
Pro Ile Asp Thr Thr Ile Met Ala Lys Asn Glu Val Phe Cys Val Gln		
1315	1320	1325
cct gag aag ggg ggt cgt aag cca gct cgt ctc atc gtg ttc ccc gat	4032	
Pro Glu Lys Gly Gly Arg Lys Pro Ala Arg Leu Ile Val Phe Pro Asp		
1330	1335	1340
ctg ggc gtg cgc gtg tgc gaa aag atg gct ttg tac gac gtg gtt aca	4080	
Leu Gly Val Arg Val Cys Glu Lys Met Ala Leu Tyr Asp Val Val Thr		
1345	1350	1355
aag ctc ccc ttg gcc gtg atg gga agc tcc tac gga ttc caa tac tca	4128	
Lys Leu Pro Leu Ala Val Met Gly Ser Ser Tyr Gly Phe Gln Tyr Ser		

1365	1370	1375	
cca gga cag cgg gtt gaa ttc ctc gtg caa gcg tgg aag tcc aag aaa Pro Gly Gln Arg Val Glu Phe Leu Val Gln Ala Trp Lys Ser Lys Lys 1380	1385	1390	4176
acc cca atg ggg ttc tcg tat gat acc cgc tgc ttt gac tcc aca gtc Thr Pro Met Gly Phe Ser Tyr Asp Thr Arg Cys Phe Asp Ser Thr Val 1395	1400	1405	4224
act gag agc gac atc cgt acg gag gca atc tac caa tgt tgt gac Thr Glu Ser Asp Ile Arg Thr Glu Ala Ile Tyr Gln Cys Cys Asp 1410	1415	1420	4272
ctc gac ccc caa gcc cgc gtg gcc atc aag tcc ctc acc gag agg ctt Leu Asp Pro Gln Ala Arg Val Ala Ile Lys Ser Leu Thr Glu Arg Leu 1425	1430	1435	4320
tat gtt ggg ggc cct ctt acc aat tca agg ggg gag aac tgc ggc tat Tyr Val Gly Pro Leu Thr Asn Ser Arg Gly Glu Asn Cys Gly Tyr 1445	1450	1455	4368
cgc agg tgc cgc gcg agc ggc gta ctg aca act agc tgt ggt aac acc Arg Arg Cys Arg Ala Ser Gly Val Leu Thr Thr Ser Cys Gly Asn Thr 1460	1465	1470	4416
ctc act tgc tac atc aag gcc cgg gca gcc tgt cga gcc gca ggg ctc Leu Thr Cys Tyr Ile Lys Ala Arg Ala Ala Cys Arg Ala Ala Gly Leu 1475	1480	1485	4464
cag gac tgc acc atg ctc gtg tgt ggc gac gac tta gtc gtt atc tgt Gln Asp Cys Thr Met Leu Val Cys Gly Asp Asp Leu Val Val Ile Cys 1490	1495	1500	4512
gaa agc gcg ggg gtc cag gag gac gcg gcg agc ctg aga gcc ttc acg Glu Ser Ala Gly Val Gln Glu Asp Ala Ala Ser Leu Arg Ala Phe Thr 1505	1510	1515	4560
gag gct atg acc agg tac tcc gcc ccc cct ggg gac ccc cca caa cca Glu Ala Met Thr Arg Tyr Ser Ala Pro Pro Gly Asp Pro Pro Gln Pro 1525	1530	1535	4608
gaa tac gac ttg gag ctc ata aca tca tgc tcc tcc aac gtg tca gtc Glu Tyr Asp Leu Glu Leu Ile Thr Ser Cys Ser Ser Asn Val Ser Val 1540	1545	1550	4656
gcc cac gac ggc gct gga aag agg gtc tac tac ctc acc cgt gac cct Ala His Asp Gly Ala Gly Lys Arg Val Tyr Tyr Leu Thr Arg Asp Pro 1555	1560	1565	4704
aca acc ccc ctc gcg aga gct gcg tgg gag aca gca aga cac act cca Thr Thr Pro Leu Ala Arg Ala Ala Trp Glu Thr Ala Arg His Thr Pro 1570	1575	1580	4752
gtc aat tcc tgg cta ggc aac ata atc atg ttt gcc ccc aca ctg tgg Val Asn Ser Trp Leu Gly Asn Ile Ile Met Phe Ala Pro Thr Leu Trp 1585	1590	1595	4800
gcg agg atg ata ctg atg acc cat ttc ttt agc gtc ctt ata gcc agg Ala Arg Met Ile Leu Met Thr His Phe Phe Ser Val Leu Ile Ala Arg 1605	1610	1615	4848

gac cag ctt gaa cag gcc ctc gat tgc gag atc tac ggg gcc tgc tac	4896																																																																																																																		
Asp Gln Leu Glu Gln Ala Leu Asp Cys Glu Ile Tyr Gly Ala Cys Tyr																																																																																																																			
1620	1625	1630		tcc ata gaa cca ctg gat cta cct cca atc att caa aga ctc cat ggc	4944	Ser Ile Glu Pro Leu Asp Leu Pro Pro Ile Ile Gln Arg Leu His Gly		1635	1640	1645		ctc agc gca ttt tca ctc cac agt tac tct cca ggt gaa atc aat agg	4992	Leu Ser Ala Phe Ser Leu His Ser Tyr Ser Pro Gly Glu Ile Asn Arg		1650	1655	1660		gtg gcc gca tgc ctc aga aaa ctt ggg gta ccg ccc ttg cga gct tgg	5040	Val Ala Ala Cys Leu Arg Lys Leu Gly Val Pro Pro Leu Arg Ala Trp		1665	1670	1675	1680	aga cac cgg gcc cgg agc gtc cgc gct agg ctt ctg gcc aga gga ggc	5088	Arg His Arg Ala Arg Ser Val Arg Ala Arg Leu Leu Ala Arg Gly Gly		1685	1690	1695		agg gct gcc ata tgt ggc aag tac ctc ttc aac tgg gca gta aga aca	5136	Arg Ala Ala Ile Cys Gly Lys Tyr Leu Phe Asn Trp Ala Val Arg Thr		1700	1705	1710		aag ctc aaa ctc act cca ata gcg gcc gct ggc cag ctg gac ttg tcc	5184	Lys Leu Lys Leu Thr Pro Ile Ala Ala Gly Gln Leu Asp Leu Ser		1715	1720	1725		ggc tgg ttc acg gct ggc tac agc ggg gga gac att tat cac agc gtg	5232	Gly Trp Phe Thr Ala Gly Tyr Ser Gly Gly Asp Ile Tyr His Ser Val		1730	1735	1740		tct cat gcc cgg ccc cgc tgg atc tgg ttt tgc cta ctc ctg ctt gct	5280	Ser His Ala Arg Pro Arg Trp Ile Trp Phe Cys Leu Leu Leu Ala		1745	1750	1755	1760	gca ggg gta ggc atc tac ctc ctc ccc aac cga atg agc acg aat cct	5328	Ala Gly Val Gly Ile Tyr Leu Leu Pro Asn Arg Met Ser Thr Asn Pro		1765	1770	1775		aaa cct caa aga aag acc aaa cgt aac acc aac cgg cgg ccg cag gac	5376	Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln Asp		1780	1785	1790		gtc aag ttc ccg ggt ggc ggt cag atc gtt ggt gga gtt tac ttg ttg	5424	Val Lys Phe Pro Gly Gly Gln Ile Val Gly Gly Val Tyr Leu Leu		1795	1800	1805		ccg cgc agg ggc cct aga ttg ggt gtg cgc gcg acg aga aag act tcc	5472	Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala Thr Arg Lys Thr Ser		1810	1815	1820		gag cgg tcg caa cct cga ggt aga cgt cag cct atc ccc aag gct cgt	5520	Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro Ile Pro Lys Ala Arg		1825	1830	1835	1840	cgccccc gag ggc agg acc tgg gct cag ccc ggg tac cct tgg ccc ctc	5568	Arg Pro Glu Gly Arg Thr Trp Ala Gln Pro Gly Tyr Pro Trp Pro Leu		1845	1850	1855	
1630																																																																																																																			
tcc ata gaa cca ctg gat cta cct cca atc att caa aga ctc cat ggc	4944																																																																																																																		
Ser Ile Glu Pro Leu Asp Leu Pro Pro Ile Ile Gln Arg Leu His Gly																																																																																																																			
1635	1640	1645		ctc agc gca ttt tca ctc cac agt tac tct cca ggt gaa atc aat agg	4992	Leu Ser Ala Phe Ser Leu His Ser Tyr Ser Pro Gly Glu Ile Asn Arg		1650	1655	1660		gtg gcc gca tgc ctc aga aaa ctt ggg gta ccg ccc ttg cga gct tgg	5040	Val Ala Ala Cys Leu Arg Lys Leu Gly Val Pro Pro Leu Arg Ala Trp		1665	1670	1675	1680	aga cac cgg gcc cgg agc gtc cgc gct agg ctt ctg gcc aga gga ggc	5088	Arg His Arg Ala Arg Ser Val Arg Ala Arg Leu Leu Ala Arg Gly Gly		1685	1690	1695		agg gct gcc ata tgt ggc aag tac ctc ttc aac tgg gca gta aga aca	5136	Arg Ala Ala Ile Cys Gly Lys Tyr Leu Phe Asn Trp Ala Val Arg Thr		1700	1705	1710		aag ctc aaa ctc act cca ata gcg gcc gct ggc cag ctg gac ttg tcc	5184	Lys Leu Lys Leu Thr Pro Ile Ala Ala Gly Gln Leu Asp Leu Ser		1715	1720	1725		ggc tgg ttc acg gct ggc tac agc ggg gga gac att tat cac agc gtg	5232	Gly Trp Phe Thr Ala Gly Tyr Ser Gly Gly Asp Ile Tyr His Ser Val		1730	1735	1740		tct cat gcc cgg ccc cgc tgg atc tgg ttt tgc cta ctc ctg ctt gct	5280	Ser His Ala Arg Pro Arg Trp Ile Trp Phe Cys Leu Leu Leu Ala		1745	1750	1755	1760	gca ggg gta ggc atc tac ctc ctc ccc aac cga atg agc acg aat cct	5328	Ala Gly Val Gly Ile Tyr Leu Leu Pro Asn Arg Met Ser Thr Asn Pro		1765	1770	1775		aaa cct caa aga aag acc aaa cgt aac acc aac cgg cgg ccg cag gac	5376	Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln Asp		1780	1785	1790		gtc aag ttc ccg ggt ggc ggt cag atc gtt ggt gga gtt tac ttg ttg	5424	Val Lys Phe Pro Gly Gly Gln Ile Val Gly Gly Val Tyr Leu Leu		1795	1800	1805		ccg cgc agg ggc cct aga ttg ggt gtg cgc gcg acg aga aag act tcc	5472	Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala Thr Arg Lys Thr Ser		1810	1815	1820		gag cgg tcg caa cct cga ggt aga cgt cag cct atc ccc aag gct cgt	5520	Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro Ile Pro Lys Ala Arg		1825	1830	1835	1840	cgccccc gag ggc agg acc tgg gct cag ccc ggg tac cct tgg ccc ctc	5568	Arg Pro Glu Gly Arg Thr Trp Ala Gln Pro Gly Tyr Pro Trp Pro Leu		1845	1850	1855									
1645																																																																																																																			
ctc agc gca ttt tca ctc cac agt tac tct cca ggt gaa atc aat agg	4992																																																																																																																		
Leu Ser Ala Phe Ser Leu His Ser Tyr Ser Pro Gly Glu Ile Asn Arg																																																																																																																			
1650	1655	1660		gtg gcc gca tgc ctc aga aaa ctt ggg gta ccg ccc ttg cga gct tgg	5040	Val Ala Ala Cys Leu Arg Lys Leu Gly Val Pro Pro Leu Arg Ala Trp		1665	1670	1675	1680	aga cac cgg gcc cgg agc gtc cgc gct agg ctt ctg gcc aga gga ggc	5088	Arg His Arg Ala Arg Ser Val Arg Ala Arg Leu Leu Ala Arg Gly Gly		1685	1690	1695		agg gct gcc ata tgt ggc aag tac ctc ttc aac tgg gca gta aga aca	5136	Arg Ala Ala Ile Cys Gly Lys Tyr Leu Phe Asn Trp Ala Val Arg Thr		1700	1705	1710		aag ctc aaa ctc act cca ata gcg gcc gct ggc cag ctg gac ttg tcc	5184	Lys Leu Lys Leu Thr Pro Ile Ala Ala Gly Gln Leu Asp Leu Ser		1715	1720	1725		ggc tgg ttc acg gct ggc tac agc ggg gga gac att tat cac agc gtg	5232	Gly Trp Phe Thr Ala Gly Tyr Ser Gly Gly Asp Ile Tyr His Ser Val		1730	1735	1740		tct cat gcc cgg ccc cgc tgg atc tgg ttt tgc cta ctc ctg ctt gct	5280	Ser His Ala Arg Pro Arg Trp Ile Trp Phe Cys Leu Leu Leu Ala		1745	1750	1755	1760	gca ggg gta ggc atc tac ctc ctc ccc aac cga atg agc acg aat cct	5328	Ala Gly Val Gly Ile Tyr Leu Leu Pro Asn Arg Met Ser Thr Asn Pro		1765	1770	1775		aaa cct caa aga aag acc aaa cgt aac acc aac cgg cgg ccg cag gac	5376	Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln Asp		1780	1785	1790		gtc aag ttc ccg ggt ggc ggt cag atc gtt ggt gga gtt tac ttg ttg	5424	Val Lys Phe Pro Gly Gly Gln Ile Val Gly Gly Val Tyr Leu Leu		1795	1800	1805		ccg cgc agg ggc cct aga ttg ggt gtg cgc gcg acg aga aag act tcc	5472	Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala Thr Arg Lys Thr Ser		1810	1815	1820		gag cgg tcg caa cct cga ggt aga cgt cag cct atc ccc aag gct cgt	5520	Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro Ile Pro Lys Ala Arg		1825	1830	1835	1840	cgccccc gag ggc agg acc tgg gct cag ccc ggg tac cct tgg ccc ctc	5568	Arg Pro Glu Gly Arg Thr Trp Ala Gln Pro Gly Tyr Pro Trp Pro Leu		1845	1850	1855																	
1660																																																																																																																			
gtg gcc gca tgc ctc aga aaa ctt ggg gta ccg ccc ttg cga gct tgg	5040																																																																																																																		
Val Ala Ala Cys Leu Arg Lys Leu Gly Val Pro Pro Leu Arg Ala Trp																																																																																																																			
1665	1670	1675	1680	aga cac cgg gcc cgg agc gtc cgc gct agg ctt ctg gcc aga gga ggc	5088	Arg His Arg Ala Arg Ser Val Arg Ala Arg Leu Leu Ala Arg Gly Gly		1685	1690	1695		agg gct gcc ata tgt ggc aag tac ctc ttc aac tgg gca gta aga aca	5136	Arg Ala Ala Ile Cys Gly Lys Tyr Leu Phe Asn Trp Ala Val Arg Thr		1700	1705	1710		aag ctc aaa ctc act cca ata gcg gcc gct ggc cag ctg gac ttg tcc	5184	Lys Leu Lys Leu Thr Pro Ile Ala Ala Gly Gln Leu Asp Leu Ser		1715	1720	1725		ggc tgg ttc acg gct ggc tac agc ggg gga gac att tat cac agc gtg	5232	Gly Trp Phe Thr Ala Gly Tyr Ser Gly Gly Asp Ile Tyr His Ser Val		1730	1735	1740		tct cat gcc cgg ccc cgc tgg atc tgg ttt tgc cta ctc ctg ctt gct	5280	Ser His Ala Arg Pro Arg Trp Ile Trp Phe Cys Leu Leu Leu Ala		1745	1750	1755	1760	gca ggg gta ggc atc tac ctc ctc ccc aac cga atg agc acg aat cct	5328	Ala Gly Val Gly Ile Tyr Leu Leu Pro Asn Arg Met Ser Thr Asn Pro		1765	1770	1775		aaa cct caa aga aag acc aaa cgt aac acc aac cgg cgg ccg cag gac	5376	Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln Asp		1780	1785	1790		gtc aag ttc ccg ggt ggc ggt cag atc gtt ggt gga gtt tac ttg ttg	5424	Val Lys Phe Pro Gly Gly Gln Ile Val Gly Gly Val Tyr Leu Leu		1795	1800	1805		ccg cgc agg ggc cct aga ttg ggt gtg cgc gcg acg aga aag act tcc	5472	Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala Thr Arg Lys Thr Ser		1810	1815	1820		gag cgg tcg caa cct cga ggt aga cgt cag cct atc ccc aag gct cgt	5520	Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro Ile Pro Lys Ala Arg		1825	1830	1835	1840	cgccccc gag ggc agg acc tgg gct cag ccc ggg tac cct tgg ccc ctc	5568	Arg Pro Glu Gly Arg Thr Trp Ala Gln Pro Gly Tyr Pro Trp Pro Leu		1845	1850	1855																									
1675	1680																																																																																																																		
aga cac cgg gcc cgg agc gtc cgc gct agg ctt ctg gcc aga gga ggc	5088																																																																																																																		
Arg His Arg Ala Arg Ser Val Arg Ala Arg Leu Leu Ala Arg Gly Gly																																																																																																																			
1685	1690	1695		agg gct gcc ata tgt ggc aag tac ctc ttc aac tgg gca gta aga aca	5136	Arg Ala Ala Ile Cys Gly Lys Tyr Leu Phe Asn Trp Ala Val Arg Thr		1700	1705	1710		aag ctc aaa ctc act cca ata gcg gcc gct ggc cag ctg gac ttg tcc	5184	Lys Leu Lys Leu Thr Pro Ile Ala Ala Gly Gln Leu Asp Leu Ser		1715	1720	1725		ggc tgg ttc acg gct ggc tac agc ggg gga gac att tat cac agc gtg	5232	Gly Trp Phe Thr Ala Gly Tyr Ser Gly Gly Asp Ile Tyr His Ser Val		1730	1735	1740		tct cat gcc cgg ccc cgc tgg atc tgg ttt tgc cta ctc ctg ctt gct	5280	Ser His Ala Arg Pro Arg Trp Ile Trp Phe Cys Leu Leu Leu Ala		1745	1750	1755	1760	gca ggg gta ggc atc tac ctc ctc ccc aac cga atg agc acg aat cct	5328	Ala Gly Val Gly Ile Tyr Leu Leu Pro Asn Arg Met Ser Thr Asn Pro		1765	1770	1775		aaa cct caa aga aag acc aaa cgt aac acc aac cgg cgg ccg cag gac	5376	Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln Asp		1780	1785	1790		gtc aag ttc ccg ggt ggc ggt cag atc gtt ggt gga gtt tac ttg ttg	5424	Val Lys Phe Pro Gly Gly Gln Ile Val Gly Gly Val Tyr Leu Leu		1795	1800	1805		ccg cgc agg ggc cct aga ttg ggt gtg cgc gcg acg aga aag act tcc	5472	Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala Thr Arg Lys Thr Ser		1810	1815	1820		gag cgg tcg caa cct cga ggt aga cgt cag cct atc ccc aag gct cgt	5520	Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro Ile Pro Lys Ala Arg		1825	1830	1835	1840	cgccccc gag ggc agg acc tgg gct cag ccc ggg tac cct tgg ccc ctc	5568	Arg Pro Glu Gly Arg Thr Trp Ala Gln Pro Gly Tyr Pro Trp Pro Leu		1845	1850	1855																																	
1695																																																																																																																			
agg gct gcc ata tgt ggc aag tac ctc ttc aac tgg gca gta aga aca	5136																																																																																																																		
Arg Ala Ala Ile Cys Gly Lys Tyr Leu Phe Asn Trp Ala Val Arg Thr																																																																																																																			
1700	1705	1710		aag ctc aaa ctc act cca ata gcg gcc gct ggc cag ctg gac ttg tcc	5184	Lys Leu Lys Leu Thr Pro Ile Ala Ala Gly Gln Leu Asp Leu Ser		1715	1720	1725		ggc tgg ttc acg gct ggc tac agc ggg gga gac att tat cac agc gtg	5232	Gly Trp Phe Thr Ala Gly Tyr Ser Gly Gly Asp Ile Tyr His Ser Val		1730	1735	1740		tct cat gcc cgg ccc cgc tgg atc tgg ttt tgc cta ctc ctg ctt gct	5280	Ser His Ala Arg Pro Arg Trp Ile Trp Phe Cys Leu Leu Leu Ala		1745	1750	1755	1760	gca ggg gta ggc atc tac ctc ctc ccc aac cga atg agc acg aat cct	5328	Ala Gly Val Gly Ile Tyr Leu Leu Pro Asn Arg Met Ser Thr Asn Pro		1765	1770	1775		aaa cct caa aga aag acc aaa cgt aac acc aac cgg cgg ccg cag gac	5376	Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln Asp		1780	1785	1790		gtc aag ttc ccg ggt ggc ggt cag atc gtt ggt gga gtt tac ttg ttg	5424	Val Lys Phe Pro Gly Gly Gln Ile Val Gly Gly Val Tyr Leu Leu		1795	1800	1805		ccg cgc agg ggc cct aga ttg ggt gtg cgc gcg acg aga aag act tcc	5472	Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala Thr Arg Lys Thr Ser		1810	1815	1820		gag cgg tcg caa cct cga ggt aga cgt cag cct atc ccc aag gct cgt	5520	Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro Ile Pro Lys Ala Arg		1825	1830	1835	1840	cgccccc gag ggc agg acc tgg gct cag ccc ggg tac cct tgg ccc ctc	5568	Arg Pro Glu Gly Arg Thr Trp Ala Gln Pro Gly Tyr Pro Trp Pro Leu		1845	1850	1855																																									
1710																																																																																																																			
aag ctc aaa ctc act cca ata gcg gcc gct ggc cag ctg gac ttg tcc	5184																																																																																																																		
Lys Leu Lys Leu Thr Pro Ile Ala Ala Gly Gln Leu Asp Leu Ser																																																																																																																			
1715	1720	1725		ggc tgg ttc acg gct ggc tac agc ggg gga gac att tat cac agc gtg	5232	Gly Trp Phe Thr Ala Gly Tyr Ser Gly Gly Asp Ile Tyr His Ser Val		1730	1735	1740		tct cat gcc cgg ccc cgc tgg atc tgg ttt tgc cta ctc ctg ctt gct	5280	Ser His Ala Arg Pro Arg Trp Ile Trp Phe Cys Leu Leu Leu Ala		1745	1750	1755	1760	gca ggg gta ggc atc tac ctc ctc ccc aac cga atg agc acg aat cct	5328	Ala Gly Val Gly Ile Tyr Leu Leu Pro Asn Arg Met Ser Thr Asn Pro		1765	1770	1775		aaa cct caa aga aag acc aaa cgt aac acc aac cgg cgg ccg cag gac	5376	Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln Asp		1780	1785	1790		gtc aag ttc ccg ggt ggc ggt cag atc gtt ggt gga gtt tac ttg ttg	5424	Val Lys Phe Pro Gly Gly Gln Ile Val Gly Gly Val Tyr Leu Leu		1795	1800	1805		ccg cgc agg ggc cct aga ttg ggt gtg cgc gcg acg aga aag act tcc	5472	Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala Thr Arg Lys Thr Ser		1810	1815	1820		gag cgg tcg caa cct cga ggt aga cgt cag cct atc ccc aag gct cgt	5520	Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro Ile Pro Lys Ala Arg		1825	1830	1835	1840	cgccccc gag ggc agg acc tgg gct cag ccc ggg tac cct tgg ccc ctc	5568	Arg Pro Glu Gly Arg Thr Trp Ala Gln Pro Gly Tyr Pro Trp Pro Leu		1845	1850	1855																																																	
1725																																																																																																																			
ggc tgg ttc acg gct ggc tac agc ggg gga gac att tat cac agc gtg	5232																																																																																																																		
Gly Trp Phe Thr Ala Gly Tyr Ser Gly Gly Asp Ile Tyr His Ser Val																																																																																																																			
1730	1735	1740		tct cat gcc cgg ccc cgc tgg atc tgg ttt tgc cta ctc ctg ctt gct	5280	Ser His Ala Arg Pro Arg Trp Ile Trp Phe Cys Leu Leu Leu Ala		1745	1750	1755	1760	gca ggg gta ggc atc tac ctc ctc ccc aac cga atg agc acg aat cct	5328	Ala Gly Val Gly Ile Tyr Leu Leu Pro Asn Arg Met Ser Thr Asn Pro		1765	1770	1775		aaa cct caa aga aag acc aaa cgt aac acc aac cgg cgg ccg cag gac	5376	Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln Asp		1780	1785	1790		gtc aag ttc ccg ggt ggc ggt cag atc gtt ggt gga gtt tac ttg ttg	5424	Val Lys Phe Pro Gly Gly Gln Ile Val Gly Gly Val Tyr Leu Leu		1795	1800	1805		ccg cgc agg ggc cct aga ttg ggt gtg cgc gcg acg aga aag act tcc	5472	Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala Thr Arg Lys Thr Ser		1810	1815	1820		gag cgg tcg caa cct cga ggt aga cgt cag cct atc ccc aag gct cgt	5520	Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro Ile Pro Lys Ala Arg		1825	1830	1835	1840	cgccccc gag ggc agg acc tgg gct cag ccc ggg tac cct tgg ccc ctc	5568	Arg Pro Glu Gly Arg Thr Trp Ala Gln Pro Gly Tyr Pro Trp Pro Leu		1845	1850	1855																																																									
1740																																																																																																																			
tct cat gcc cgg ccc cgc tgg atc tgg ttt tgc cta ctc ctg ctt gct	5280																																																																																																																		
Ser His Ala Arg Pro Arg Trp Ile Trp Phe Cys Leu Leu Leu Ala																																																																																																																			
1745	1750	1755	1760	gca ggg gta ggc atc tac ctc ctc ccc aac cga atg agc acg aat cct	5328	Ala Gly Val Gly Ile Tyr Leu Leu Pro Asn Arg Met Ser Thr Asn Pro		1765	1770	1775		aaa cct caa aga aag acc aaa cgt aac acc aac cgg cgg ccg cag gac	5376	Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln Asp		1780	1785	1790		gtc aag ttc ccg ggt ggc ggt cag atc gtt ggt gga gtt tac ttg ttg	5424	Val Lys Phe Pro Gly Gly Gln Ile Val Gly Gly Val Tyr Leu Leu		1795	1800	1805		ccg cgc agg ggc cct aga ttg ggt gtg cgc gcg acg aga aag act tcc	5472	Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala Thr Arg Lys Thr Ser		1810	1815	1820		gag cgg tcg caa cct cga ggt aga cgt cag cct atc ccc aag gct cgt	5520	Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro Ile Pro Lys Ala Arg		1825	1830	1835	1840	cgccccc gag ggc agg acc tgg gct cag ccc ggg tac cct tgg ccc ctc	5568	Arg Pro Glu Gly Arg Thr Trp Ala Gln Pro Gly Tyr Pro Trp Pro Leu		1845	1850	1855																																																																	
1755	1760																																																																																																																		
gca ggg gta ggc atc tac ctc ctc ccc aac cga atg agc acg aat cct	5328																																																																																																																		
Ala Gly Val Gly Ile Tyr Leu Leu Pro Asn Arg Met Ser Thr Asn Pro																																																																																																																			
1765	1770	1775		aaa cct caa aga aag acc aaa cgt aac acc aac cgg cgg ccg cag gac	5376	Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln Asp		1780	1785	1790		gtc aag ttc ccg ggt ggc ggt cag atc gtt ggt gga gtt tac ttg ttg	5424	Val Lys Phe Pro Gly Gly Gln Ile Val Gly Gly Val Tyr Leu Leu		1795	1800	1805		ccg cgc agg ggc cct aga ttg ggt gtg cgc gcg acg aga aag act tcc	5472	Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala Thr Arg Lys Thr Ser		1810	1815	1820		gag cgg tcg caa cct cga ggt aga cgt cag cct atc ccc aag gct cgt	5520	Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro Ile Pro Lys Ala Arg		1825	1830	1835	1840	cgccccc gag ggc agg acc tgg gct cag ccc ggg tac cct tgg ccc ctc	5568	Arg Pro Glu Gly Arg Thr Trp Ala Gln Pro Gly Tyr Pro Trp Pro Leu		1845	1850	1855																																																																									
1775																																																																																																																			
aaa cct caa aga aag acc aaa cgt aac acc aac cgg cgg ccg cag gac	5376																																																																																																																		
Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln Asp																																																																																																																			
1780	1785	1790		gtc aag ttc ccg ggt ggc ggt cag atc gtt ggt gga gtt tac ttg ttg	5424	Val Lys Phe Pro Gly Gly Gln Ile Val Gly Gly Val Tyr Leu Leu		1795	1800	1805		ccg cgc agg ggc cct aga ttg ggt gtg cgc gcg acg aga aag act tcc	5472	Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala Thr Arg Lys Thr Ser		1810	1815	1820		gag cgg tcg caa cct cga ggt aga cgt cag cct atc ccc aag gct cgt	5520	Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro Ile Pro Lys Ala Arg		1825	1830	1835	1840	cgccccc gag ggc agg acc tgg gct cag ccc ggg tac cct tgg ccc ctc	5568	Arg Pro Glu Gly Arg Thr Trp Ala Gln Pro Gly Tyr Pro Trp Pro Leu		1845	1850	1855																																																																																	
1790																																																																																																																			
gtc aag ttc ccg ggt ggc ggt cag atc gtt ggt gga gtt tac ttg ttg	5424																																																																																																																		
Val Lys Phe Pro Gly Gly Gln Ile Val Gly Gly Val Tyr Leu Leu																																																																																																																			
1795	1800	1805		ccg cgc agg ggc cct aga ttg ggt gtg cgc gcg acg aga aag act tcc	5472	Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala Thr Arg Lys Thr Ser		1810	1815	1820		gag cgg tcg caa cct cga ggt aga cgt cag cct atc ccc aag gct cgt	5520	Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro Ile Pro Lys Ala Arg		1825	1830	1835	1840	cgccccc gag ggc agg acc tgg gct cag ccc ggg tac cct tgg ccc ctc	5568	Arg Pro Glu Gly Arg Thr Trp Ala Gln Pro Gly Tyr Pro Trp Pro Leu		1845	1850	1855																																																																																									
1805																																																																																																																			
ccg cgc agg ggc cct aga ttg ggt gtg cgc gcg acg aga aag act tcc	5472																																																																																																																		
Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala Thr Arg Lys Thr Ser																																																																																																																			
1810	1815	1820		gag cgg tcg caa cct cga ggt aga cgt cag cct atc ccc aag gct cgt	5520	Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro Ile Pro Lys Ala Arg		1825	1830	1835	1840	cgccccc gag ggc agg acc tgg gct cag ccc ggg tac cct tgg ccc ctc	5568	Arg Pro Glu Gly Arg Thr Trp Ala Gln Pro Gly Tyr Pro Trp Pro Leu		1845	1850	1855																																																																																																	
1820																																																																																																																			
gag cgg tcg caa cct cga ggt aga cgt cag cct atc ccc aag gct cgt	5520																																																																																																																		
Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro Ile Pro Lys Ala Arg																																																																																																																			
1825	1830	1835	1840	cgccccc gag ggc agg acc tgg gct cag ccc ggg tac cct tgg ccc ctc	5568	Arg Pro Glu Gly Arg Thr Trp Ala Gln Pro Gly Tyr Pro Trp Pro Leu		1845	1850	1855																																																																																																									
1835	1840																																																																																																																		
cgccccc gag ggc agg acc tgg gct cag ccc ggg tac cct tgg ccc ctc	5568																																																																																																																		
Arg Pro Glu Gly Arg Thr Trp Ala Gln Pro Gly Tyr Pro Trp Pro Leu																																																																																																																			
1845	1850	1855																																																																																																																	
1855																																																																																																																			

tat ggc aat gag ggc tgc ggg tgg gcg gga tgg ctc ctg tct ccc cgt	5616
Tyr Gly Asn Glu Gly Cys Gly Trp Ala Gly Trp Leu Leu Ser Pro Arg	
1860 1865 1870	
ggc tct cgg cct agc tgg ggc ccc aca gac ccc cgg cgt agg tcg cgc	5664
Gly Ser Arg Pro Ser Trp Gly Pro Thr Asp Pro Arg Arg Arg Ser Arg	
1875 1880 1885	
aat ttg ggt aag	5676
Asn Leu Gly Lys	
1890	
<210> 8	
<211> 1892	
<212> PRT	
<213> Artificial Sequence	
<220>	
<223> Description of Artificial Sequence: representative	
NS345Core fusion protein	
<400> 8	
Met Ala Ala Tyr Ala Ala Gln Gly Tyr Lys Val Leu Val Leu Asn Pro	
1 5 10 15	
Ser Val Ala Ala Thr Leu Gly Phe Gly Ala Tyr Met Ser Lys Ala His	
20 25 30	
Gly Ile Asp Pro Asn Ile Arg Thr Gly Val Arg Thr Ile Thr Thr Gly	
35 40 45	
Ser Pro Ile Thr Tyr Ser Thr Tyr Gly Lys Phe Leu Ala Asp Gly Gly	
50 55 60	
Cys Ser Gly Gly Ala Tyr Asp Ile Ile Cys Asp Glu Cys His Ser	
65 70 75 80	
Thr Asp Ala Thr Ser Ile Leu Gly Ile Gly Thr Val Leu Asp Gln Ala	
85 90 95	
Glu Thr Ala Gly Ala Arg Leu Val Val Leu Ala Thr Ala Thr Pro Pro	
100 105 110	
Gly Ser Val Thr Val Pro His Pro Asn Ile Glu Glu Val Ala Leu Ser	
115 120 125	
Thr Thr Gly Glu Ile Pro Phe Tyr Gly Lys Ala Ile Pro Leu Glu Val	
130 135 140	
Ile Lys Gly Gly Arg His Leu Ile Phe Cys His Ser Lys Lys Lys Cys	
145 150 155 160	
Asp Glu Leu Ala Ala Lys Leu Val Ala Leu Gly Ile Asn Ala Val Ala	
165 170 175	
Tyr Tyr Arg Gly Leu Asp Val Ser Val Ile Pro Thr Ser Gly Asp Val	
180 185 190	
Val Val Val Ala Thr Asp Ala Leu Met Thr Gly Tyr Thr Gly Asp Phe	
195 200 205	

Asp Ser Val Ile Asp Cys Asn Thr Cys Val Thr Gln Thr Val Asp Phe
 210 215 220

Ser Leu Asp Pro Thr Phe Thr Ile Glu Thr Ile Thr Leu Pro Gln Asp
 225 230 235 240

Ala Val Ser Arg Thr Gln Arg Arg Gly Arg Thr Gly Arg Gly Lys Pro
 245 250 255

Gly Ile Tyr Arg Phe Val Ala Pro Gly Glu Arg Pro Ser Gly Met Phe
 260 265 270

Asp Ser Ser Val Leu Cys Glu Cys Tyr Asp Ala Gly Cys Ala Trp Tyr
 275 280 285

Glu Leu Thr Pro Ala Glu Thr Thr Val Arg Leu Arg Ala Tyr Met Asn
 290 295 300

Thr Pro Gly Leu Pro Val Cys Gln Asp His Leu Glu Phe Trp Glu Gly
 305 310 315 320

Val Phe Thr Gly Leu Thr His Ile Asp Ala His Phe Leu Ser Gln Thr
 325 330 335

Lys Gln Ser Gly Glu Asn Leu Pro Tyr Leu Val Ala Tyr Gln Ala Thr
 340 345 350

Val Cys Ala Arg Ala Gln Ala Pro Pro Pro Ser Trp Asp Gln Met Trp
 355 360 365

Lys Cys Leu Ile Arg Leu Lys Pro Thr Leu His Gly Pro Thr Pro Leu
 370 375 380

Leu Tyr Arg Leu Gly Ala Val Gln Asn Glu Ile Thr Leu Thr His Pro
 385 390 395 400

Val Thr Lys Tyr Ile Met Thr Cys Met Ser Ala Asp Leu Glu Val Val
 405 410 415

Thr Ser Thr Trp Val Leu Val Gly Gly Val Leu Ala Ala Leu Ala Ala
 420 425 430

Tyr Cys Leu Ser Thr Gly Cys Val Val Ile Val Gly Arg Val Val Leu
 435 440 445

Ser Gly Lys Pro Ala Ile Ile Pro Asp Arg Glu Val Leu Tyr Arg Glu
 450 455 460

Phe Asp Glu Met Glu Glu Cys Ser Gln His Leu Pro Tyr Ile Glu Gln
 465 470 475 480

Gly Met Met Leu Ala Glu Gln Phe Lys Gln Lys Ala Leu Gly Leu Leu
 485 490 495

Gln Thr Ala Ser Arg Gln Ala Glu Val Ile Ala Pro Ala Val Gln Thr
 500 505 510

Asn Trp Gln Lys Leu Glu Thr Phe Trp Ala Lys His Met Trp Asn Phe
 515 520 525

Ile Ser Gly Ile Gln Tyr Leu Ala Gly Leu Ser Thr Leu Pro Gly Asn
 530 535 540
 Pro Ala Ile Ala Ser Leu Met Ala Phe Thr Ala Ala Val Thr Ser Pro
 545 550 555 560
 Leu Thr Thr Ser Gln Thr Leu Leu Phe Asn Ile Leu Gly Gly Trp Val
 565 570 575
 Ala Ala Gln Leu Ala Ala Pro Gly Ala Ala Thr Ala Phe Val Gly Ala
 580 585 590
 Gly Leu Ala Gly Ala Ala Ile Gly Ser Val Gly Leu Gly Lys Val Leu
 595 600 605
 Ile Asp Ile Leu Ala Gly Tyr Gly Ala Gly Val Ala Gly Ala Leu Val
 610 615 620
 Ala Phe Lys Ile Met Ser Gly Glu Val Pro Ser Thr Glu Asp Leu Val
 625 630 635 640
 Asn Leu Leu Pro Ala Ile Leu Ser Pro Gly Ala Leu Val Val Gly Val
 645 650 655
 Val Cys Ala Ala Ile Leu Arg Arg His Val Gly Pro Gly Glu Gly Ala
 660 665 670
 Val Gln Trp Met Asn Arg Leu Ile Ala Phe Ala Ser Arg Gly Asn His
 675 680 685
 Val Ser Pro Thr His Tyr Val Pro Glu Ser Asp Ala Ala Arg Val
 690 695 700
 Thr Ala Ile Leu Ser Ser Leu Thr Val Thr Gln Leu Leu Arg Arg Leu
 705 710 715 720
 His Gln Trp Ile Ser Ser Glu Cys Thr Thr Pro Cys Ser Gly Ser Trp
 725 730 735
 Leu Arg Asp Ile Trp Asp Trp Ile Cys Glu Val Leu Ser Asp Phe Lys
 740 745 750
 Thr Trp Leu Lys Ala Lys Leu Met Pro Gln Leu Pro Gly Ile Pro Phe
 755 760 765
 Val Ser Cys Gln Arg Gly Tyr Lys Gly Val Trp Arg Gly Asp Gly Ile
 770 775 780
 Met His Thr Arg Cys His Cys Gly Ala Glu Ile Thr Gly His Val Lys
 785 790 795 800
 Asn Gly Thr Met Arg Ile Val Gly Pro Arg Thr Cys Arg Asn Met Trp
 805 810 815
 Ser Gly Thr Phe Pro Ile Asn Ala Tyr Thr Thr Gly Pro Cys Thr Pro
 820 825 830
 Leu Pro Ala Pro Asn Tyr Thr Phe Ala Leu Trp Arg Val Ser Ala Glu
 835 840 845
 Glu Tyr Val Glu Ile Arg Gln Val Gly Asp Phe His Tyr Val Thr Gly

850	855	860
Met Thr Thr Asp Asn Leu Lys Cys Pro Cys Gln Val Pro Ser Pro Glu		
865	870	875
Phe Phe Thr Glu Leu Asp Gly Val Arg Leu His Arg Phe Ala Pro Pro		
885	890	895
Cys Lys Pro Leu Leu Arg Glu Glu Val Ser Phe Arg Val Gly Leu His		
900	905	910
Glu Tyr Pro Val Gly Ser Gln Leu Pro Cys Glu Pro Glu Pro Asp Val		
915	920	925
Ala Val Leu Thr Ser Met Leu Thr Asp Pro Ser His Ile Thr Ala Glu		
930	935	940
Ala Ala Gly Arg Arg Leu Ala Arg Gly Ser Pro Pro Ser Val Ala Ser		
945	950	955
Ser Ser Ala Ser Gln Leu Ser Ala Pro Ser Leu Lys Ala Thr Cys Thr		
965	970	975
Ala Asn His Asp Ser Pro Asp Ala Glu Leu Ile Glu Ala Asn Leu Leu		
980	985	990
Trp Arg Gln Glu Met Gly Gly Asn Ile Thr Arg Val Glu Ser Glu Asn		
995	1000	1005
Lys Val Val Ile Leu Asp Ser Phe Asp Pro Leu Val Ala Glu Glu Asp		
1010	1015	1020
Glu Arg Glu Ile Ser Val Pro Ala Glu Ile Leu Arg Lys Ser Arg Arg		
1025	1030	1035
Phe Ala Gln Ala Leu Pro Val Trp Ala Arg Pro Asp Tyr Asn Pro Pro		
1045	1050	1055
Leu Val Glu Thr Trp Lys Lys Pro Asp Tyr Glu Pro Pro Val Val His		
1060	1065	1070
Gly Cys Pro Leu Pro Pro Lys Ser Pro Pro Val Pro Pro Pro Arg		
1075	1080	1085
Lys Lys Arg Thr Val Val Leu Thr Glu Ser Thr Leu Ser Thr Ala Leu		
1090	1095	1100
Ala Glu Leu Ala Thr Arg Ser Phe Gly Ser Ser Ser Thr Ser Gly Ile		
1105	1110	1115
Thr Gly Asp Asn Thr Thr Ser Ser Glu Pro Ala Pro Ser Gly Cys		
1125	1130	1135
Pro Pro Asp Ser Asp Ala Glu Ser Tyr Ser Ser Met Pro Pro Leu Glu		
1140	1145	1150
Gly Glu Pro Gly Asp Pro Asp Leu Ser Asp Gly Ser Trp Ser Thr Val		
1155	1160	1165
Ser Ser Glu Ala Asn Ala Glu Asp Val Val Cys Cys Ser Met Ser Tyr		
1170	1175	1180

Ser Trp Thr Gly Ala Leu Val Thr Pro Cys Ala Ala Glu Glu Gln Lys
 1185 1190 1195 1200
 Leu Pro Ile Asn Ala Leu Ser Asn Ser Leu Leu Arg His His Asn Leu
 1205 1210 1215
 Val Tyr Ser Thr Thr Ser Arg Ser Ala Cys Gln Arg Gln Lys Lys Val
 1220 1225 1230
 Thr Phe Asp Arg Leu Gln Val Leu Asp Ser His Tyr Gln Asp Val Leu
 1235 1240 1245
 Lys Glu Val Lys Ala Ala Ala Ser Lys Val Lys Ala Asn Leu Leu Ser
 1250 1255 1260
 Val Glu Glu Ala Cys Ser Leu Thr Pro Pro His Ser Ala Lys Ser Lys
 1265 1270 1275 1280
 Phe Gly Tyr Gly Ala Lys Asp Val Arg Cys His Ala Arg Lys Ala Val
 1285 1290 1295
 Thr His Ile Asn Ser Val Trp Lys Asp Leu Leu Glu Asp Asn Val Thr
 1300 1305 1310
 Pro Ile Asp Thr Thr Ile Met Ala Lys Asn Glu Val Phe Cys Val Gln
 1315 1320 1325
 Pro Glu Lys Gly Gly Arg Lys Pro Ala Arg Leu Ile Val Phe Pro Asp
 1330 1335 1340
 Leu Gly Val Arg Val Cys Glu Lys Met Ala Leu Tyr Asp Val Val Thr
 1345 1350 1355 1360
 Lys Leu Pro Leu Ala Val Met Gly Ser Ser Tyr Gly Phe Gln Tyr Ser
 1365 1370 1375
 Pro Gly Gln Arg Val Glu Phe Leu Val Gln Ala Trp Lys Ser Lys Lys
 1380 1385 1390
 Thr Pro Met Gly Phe Ser Tyr Asp Thr Arg Cys Phe Asp Ser Thr Val
 1395 1400 1405
 Thr Glu Ser Asp Ile Arg Thr Glu Glu Ala Ile Tyr Gln Cys Cys Asp
 1410 1415 1420
 Leu Asp Pro Gln Ala Arg Val Ala Ile Lys Ser Leu Thr Glu Arg Leu
 1425 1430 1435 1440
 Tyr Val Gly Gly Pro Leu Thr Asn Ser Arg Gly Glu Asn Cys Gly Tyr
 1445 1450 1455
 Arg Arg Cys Arg Ala Ser Gly Val Leu Thr Thr Ser Cys Gly Asn Thr
 1460 1465 1470
 Leu Thr Cys Tyr Ile Lys Ala Arg Ala Ala Cys Arg Ala Ala Gly Leu
 1475 1480 1485
 Gln Asp Cys Thr Met Leu Val Cys Gly Asp Asp Leu Val Val Ile Cys
 1490 1495 1500
 Glu Ser Ala Gly Val Gln Glu Asp Ala Ala Ser Leu Arg Ala Phe Thr

1505 1510 1515 1520
 Glu Ala Met Thr Arg Tyr Ser Ala Pro Pro Gly Asp Pro Pro Gln Pro
 1525 1530 1535

 Glu Tyr Asp Leu Glu Leu Ile Thr Ser Cys Ser Ser Asn Val Ser Val
 1540 1545 1550

 Ala His Asp Gly Ala Gly Lys Arg Val Tyr Tyr Leu Thr Arg Asp Pro
 1555 1560 1565

 Thr Thr Pro Leu Ala Arg Ala Ala Trp Glu Thr Ala Arg His Thr Pro
 1570 1575 1580

 Val Asn Ser Trp Leu Gly Asn Ile Ile Met Phe Ala Pro Thr Leu Trp
 1585 1590 1595 1600

 Ala Arg Met Ile Leu Met Thr His Phe Phe Ser Val Leu Ile Ala Arg
 1605 1610 1615

 Asp Gln Leu Glu Gln Ala Leu Asp Cys Glu Ile Tyr Gly Ala Cys Tyr
 1620 1625 1630

 Ser Ile Glu Pro Leu Asp Leu Pro Pro Ile Ile Gln Arg Leu His Gly
 1635 1640 1645

 Leu Ser Ala Phe Ser Leu His Ser Tyr Ser Pro Gly Glu Ile Asn Arg
 1650 1655 1660

 Val Ala Ala Cys Leu Arg Lys Leu Gly Val Pro Pro Leu Arg Ala Trp
 1665 1670 1675 1680

 Arg His Arg Ala Arg Ser Val Arg Ala Arg Leu Leu Ala Arg Gly Gly
 1685 1690 1695

 Arg Ala Ala Ile Cys Gly Lys Tyr Leu Phe Asn Trp Ala Val Arg Thr
 1700 1705 1710

 Lys Leu Lys Leu Thr Pro Ile Ala Ala Gly Gln Leu Asp Leu Ser
 1715 1720 1725

 Gly Trp Phe Thr Ala Gly Tyr Ser Gly Gly Asp Ile Tyr His Ser Val
 1730 1735 1740

 Ser His Ala Arg Pro Arg Trp Ile Trp Phe Cys Leu Leu Leu Ala
 1745 1750 1755 1760

 Ala Gly Val Gly Ile Tyr Leu Leu Pro Asn Arg Met Ser Thr Asn Pro
 1765 1770 1775

 Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln Asp
 1780 1785 1790

 Val Lys Phe Pro Gly Gly Gln Ile Val Gly Gly Val Tyr Leu Leu
 1795 1800 1805

 Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala Thr Arg Lys Thr Ser
 1810 1815 1820

 Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro Ile Pro Lys Ala Arg
 1825 1830 1835 1840

Arg Pro Glu Gly Arg Thr Trp Ala Gln Pro Gly Tyr Pro Trp Pro Leu
 1845 1850 1855

Tyr Gly Asn Glu Gly Cys Gly Trp Ala Gly Trp Leu Leu Ser Pro Arg
 1860 1865 1870

Gly Ser Arg Pro Ser Trp Gly Pro Thr Asp Pro Arg Arg Arg Ser Arg
 1875 1880 1885

Asn Leu Gly Lys
 1890

<210> 9
 <211> 546
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: representative
 native NS3 protease domain

<220>
 <221> CDS
 <222> (1)..(546)

<400> 9
 atg gcg ccc atc acg gcg tac gcc cag cag aca agg ggc ctc cta ggg 48
 Met Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu Gly
 1 5 10 15

tgc ata atc acc agc cta act ggc cgg gac aaa aac caa gtg gag ggt 96
 Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly
 20 25 30

gag gtc cag att gtg tca act gct gcc caa acc ttc ctg gca acg tgc 144
 Glu Val Gln Ile Val Ser Thr Ala Ala Gln Thr Phe Leu Ala Thr Cys
 35 40 45

atc aat ggg gtg tgc tgg act gtc tac cac ggg gcc gga acg agg acc 192
 Ile Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Thr Arg Thr
 50 55 60

atc gcg tca ccc aag ggt cct gtc atc cag atg tat acc aat gta gac 240
 Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Met Tyr Thr Asn Val Asp
 65 70 75 80

caa gac ctt gtg ggc tgg ccc gct ccg caa ggt agc cga tca ttg aca 288
 Gln Asp Leu Val Gly Trp Pro Ala Pro Gln Gly Ser Arg Ser Leu Thr
 85 90 95

ccc tgc act tgc ggc tcc tcg gac ctt tac ctg gtc acg agg cac gcc 336
 Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His Ala
 100 105 110

gat gtc att ccc gtg cgc cgg cgg ggt gat agc agg ggc agc ctg ctg 384
 Asp Val Ile Pro Val Arg Arg Gly Asp Ser Arg Gly Ser Leu Leu
 115 120 125

tcg ccc cgg ccc att tcc tac ttg aaa ggc tcc tcg ggg ggt ccg ctg 432
 Ser Pro Arg Pro Ile Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro Leu

130	135	140	
ttg tgc ccc gcg ggg cac gcc gtg ggc ata ttt agg gcc gcg gtg tgc Leu Cys Pro Ala Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys 145 150 155 160			480
acc cgt gga gtg gct aag gcg gtg gac ttt atc cct gtg gag aac cta Thr Arg Gly Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu Asn Leu 165 170 175			528
gag aca acc atg agg tcc Glu Thr Thr Met Arg Ser 180			546
<p><210> 10 <211> 182 <212> PRT <213> Artificial Sequence</p> <p><220> <223> Description of Artificial Sequence: representative native NS3 protease domain</p>			
<p><400> 10 Met Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu Gly 1 5 10 15</p> <p>Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly 20 25 30</p> <p>Glu Val Gln Ile Val Ser Thr Ala Ala Gln Thr Phe Leu Ala Thr Cys 35 40 45</p> <p>Ile Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Thr Arg Thr 50 55 60</p> <p>Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Met Tyr Thr Asn Val Asp 65 70 75 80</p> <p>Gln Asp Leu Val Gly Trp Pro Ala Pro Gln Gly Ser Arg Ser Leu Thr 85 90 95</p> <p>Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His Ala 100 105 110</p> <p>Asp Val Ile Pro Val Arg Arg Gly Asp Ser Arg Gly Ser Leu Leu 115 120 125</p> <p>Ser Pro Arg Pro Ile Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro Leu 130 135 140</p> <p>Leu Cys Pro Ala Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys 145 150 155 160</p> <p>Thr Arg Gly Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu Asn Leu 165 170 175</p> <p>Glu Thr Thr Met Arg Ser 180</p>			